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**APPLICATION NUMBER: 60/557,290**

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| <input type="checkbox"/> Additional inventors are being named on the __ separately numbered sheets attached hereto  |  |   |           |   |                  |
| TITLE OF THE INVENTION (280 characters max)   |  |   |           |   |                  |
| NEW NANOSHELLS AND DISCRETE POLYMER-COATED NANOSHELLS, METHODS FOR MAKING AND USING SAME  |  |   |           |   |                  |
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| ENCLOSED APPLICATION PARTS (check all that apply)   |  |   |           |   |                  |
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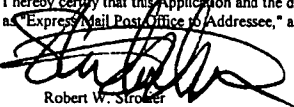
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Re: US PSN: 60/ ; FD:3/29/04  
Title: NEW NANOSHELLS AND DISCRETE POLYMER-COATED  
NANOSHELLS, METHODS FOR MAKING AND USING SAME  
Our Ref. No.: 96605/28PRV

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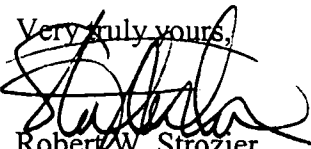
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**UNITED STATES PROVISIONAL PATENT APPLICATION**

**TITLE: NEW NANOSHELLS AND DISCRETE POLYMER-COATED NANOSHELLS, METHODS FOR MAKING AND USING SAME**

**INVENTORS: T. Randall Lee of Houston, TX and Jun-Hyun Kim of Houston, TX**

**ASSIGNEE: THE UNIVERSITY OF HOUSTON SYSTEM**

**BACKGROUND OF THE INVENTION**

**1. Field of the Invention**

[0001] The present invention relates to the general field of nanoshells.

[0002] More particularly, the present invention relates to improved nanoshells allowing for greater fields of use including, but not limited, to drug delivery, therapeutics, and electronic applications.

**2. Description of the Related Art**

[0003] It is known that solid metal nanoparticles (*i.e.*, solid, single metal spheres of uniform composition and nanometer dimensions) possess unique optical properties. In particular, metal nanoparticles (especially the coinage metals) display a pronounced optical resonance. This so-called plasmon resonance is due to the collective coupling of the conduction electrons in the metal sphere to the incident electromagnetic field. This resonance can be dominated by absorption or scattering depending on the radius of the nanoparticle with respect to the wavelength of the incident electromagnetic radiation. Associated with this plasmon resonance is a strong local field enhancement in the interior of the metal nanoparticle. A variety of potentially useful devices can be fabricated to take advantage of these specific optical properties.

[0004] Metal colloids have a variety of useful optical properties including a strong optical absorption and an extremely large and fast third-order nonlinear optical (NLO) polarizability. These optical properties are attributed to the phasic response of electrons in the metallic particles to electromagnetic fields. This collective electron excitation is known as plasmon resonance.

[0005] At resonance, dilute metal colloid solutions have the largest electronic NLO susceptibility of known substances. However, the utility of these solutions is limited because their plasmon resonance is confined to relatively narrow wavelength ranges and cannot readily be shifted. For example, silver particles 10 nm in diameter absorb light maximally at approximately 390 nm, while similar sized gold particles absorb maximally at about 520 nm. These absorbance maximums are insensitive to changes in particle size and various dielectric coatings on the particles.

[0006] A serious practical limitation to realizing many applications of solid metal nanoparticles is the inability to position the plasmon resonance at technologically important wavelengths. For example, solid gold nanoparticles of 10 nm in diameter have a plasmon resonance centered at 520 nm. This plasmon resonance cannot be controllably shifted by more than approximately 30 nanometers by varying the particle diameter or the specific embedding medium.

[0007] One method of overcoming this problem is to coat small non-conducting particles with these metals. Researchers have developed methods and materials outlining the synthesis of the composite particles having homogenous structures and defined wavelength absorbance maxima. Additional information detailing work concerning the methods of preparation of metal nanoshells can be found in U.S. Patent Nos. 6,344,272 and 6,685,986, incorporated herein by reference. In essence nanoshell composites are particles that have two layers. One layer is immediately adjacent to and surrounds another layer. The innermost layer is said to be the core. The layer surrounding the core is said to be the shell layer. The shell is metal-like in that it can conduct electricity and is made of a metal or metal-like material. The relative thickness or depth of each particles constituent layers determines the wavelength of its absorption. Therefore, by adjusting the relative core, shell thickness, and choice of materials nanoshells may be fabricated that will absorb or scatter light at any wavelength across much of the ultraviolet (UV), visible and infrared (IR) range of the electromagnetic spectrum.

[0008] The spectral location of the maximum of the plasmon resonance peak for this geometry depends sensitively upon the ratio of the core radius to shell thickness, as well as the dielectric functions of the core and shell. The presence of a dielectric core shifts the plasmon resonance to longer wavelengths relative to a solid nanoparticle made exclusively of the metallic shell material. For a given core radius, a thin shell will have a plasmon peak that is shifted to longer wavelengths relative to a thicker shell. It is to be emphasized that metal nanoshells possess all of the same technologically viable optical properties as solid metal nanoparticles in addition to this extremely important aspect of resonance tunability.

[0009] As described in the U.S. Patent 6,344,272, the nanoshells are preferably made by modifying the surface of a silica particle (the core) with aminopropyltriethoxysilane to add amine groups to the surface. These are then seeded with colloidal gold. Additional colloidal gold is added via chemical reduction in solution, to form the particle's gold shell layer. The wavelength of maximum optical absorption ( $\lambda_{max}$ ) of a particle is determined by the ratio of the core radius to the shell thickness for a particle of given core and shell materials and particle diameter. Each of these variables (*i.e.*, core

radius and shell thickness) can be easily and independently controlled during fabrication of the nanoshells. Varying the shell thickness, core diameter, and the total nanoparticle diameter allows the optical properties of the nanoshells to be tuned over the visible and near-IR spectrum. By also varying the core and shell materials, which are preferably gold or silver over a silicon dioxide or Au<sub>2</sub>S core, the tunable range can be extended to cover most of the UV to near-infrared spectrum. Thus, the optical extinction profiles of the nanoshells can be modified so that the nanoshells optimally absorb light emitted from various lasers.

[0010] With the advent of nanoshell technology, it was soon realized that tunable nanoshells would have a wide range of uses including energy efficient paints, windows, coatings, fabrics, vehicles, building structures, and in photovoltaic applications. Use of tunable nanoshells has also been applied to modulated drug-delivery applications.

[0011] Modulated drug delivery allows the release profiles of therapeutic agents to be manipulated to match the physiological requirements of the patient. This type of controlled delivery system is useful for treating diseases that affect the homeostatic functions of the body, such as diabetes mellitus. Various methods of accomplishing modulated *in vivo* drug delivery have been described in the literature and are currently in use or undergoing investigation. Methods involving sequestration of various therapeutic agents by a polymer matrix material have been examined. For example, U.S. Pat. No. 5,986,043, incorporated herein by reference, describes certain biodegradable hydrogels as carriers for biologically active materials such as hormones, enzymes, antibiotics, antineoplastic agents, and cell suspensions. Delivery of the sequestered drug depends on the *in vivo* degradation characteristics of the carrier.

[0012] Certain temperature-sensitive hydrophilic polymer gels, or hydrogels, have been described. When the temperature of the polymer is raised above its lower critical (or consolute) solution temperature (LCST), the hydrogel undergoes a reversible phase transition that results in the collapse of the hydrogel structure (A. S. Hoffman et al. J. Contr. Rel. 4:213-222 (1986); and L. C. Dong et al. J. Contr. Rel. 4:223-227 (1986)). The hydrogel collapse forces soluble materials held within the hydrogel matrix to be expelled into the surrounding solution (R. Yoshida et al. J. Biomater. Sci. Polymer Edn. 6:585-598 (1994)). An impediment in the development of temperature-sensitive materials into clinically useful modulated drug-delivery devices has been the lack of satisfactory means for altering the temperature of the implanted device. Ideally, the temperature change should be localized to the device to avoid damage to surrounding tissue, but the temperature change also

must be rapid in order to control the conformational changes in the polymer and the drug delivery profile. Other means of altering the temperature have been proposed and are being investigated, such as heating pads, non-targeted light and exothermic chemical reactions. Other proposed techniques for controlled drug release include the application of alternating magnetic fields to certain polymers with embedded magnetic particles to effect modulation of drug delivery.

[0013] Presently an available method offering a satisfactory way of obtaining localized heating to accomplish controlled, thermally actuated drug release from implantable nanoshells while adequately avoiding potential damage to the surrounding body tissue is described in U.S. Patent No. 6,645,517, incorporated herein by reference. The technology allows for nanoshells as employers of heat-transfer agents that are embedded within a hydrogel polymer matrix. As the near-IR light is absorbed by the nanoshells, heat is generated and transferred to the polymer matrix nearby. As a result, the temperature of the polymer is increased above the polymer's lower critical solution temperature (LCST), causing a conformational change in the copolymer that leads to alterations in the release profile of the entrapped drug.

[0014] Improved nanoshells based upon decreased particle size, shell thickness and uniformity are needed for use in broader applications such as electronics and medical applications.

### **SUMMARY OF THE INVENTION**

[0015] The present invention relates to improved oxide or metal oxide cores, where the core may be comprised of alumina, silica, aluminosilicate, silicaaluminate, zirconia, titania, mangnesia, or other similar oxides and mixtures or combinations thereof having noble metal shells, where the shells are an alloy, where the alloy allows for the formation of more uniform and thinner shells so that the resulting nanoshells have improved optical characteristics. The method for making the shells improves structure, size, and optical properties.

[0016] The present invention relates to metallic nanoparticles such as, but not limited to, noble metal nanoparticles (preferably silver (Ag) nanoparticles) having deposited thereon a shell of a noble metal or noble metal alloy such as, but not limited to, Group VIB, VIIB, VIII metals and the noble metals (Cr, Mo, W, Fe, Co, Ni, Cu, Zn, Ru, Rh, Pd, Ag, Cd, Os, Ir, Pt, Au), with special emphasis on Ru, Rh, Pd, Ag, Os, Ir, Pt, and Au.

[0017] The present invention relates to metallic nanoparticles such as, but not limited to, noble metal or alloy nanoparticles having deposited thereon a shell of a metal alloy having a plasmon resonance. These alloy-coated metal core particles can be made of a smaller particle size than their silica core



counterparts, lending them improved properties including optical properties for use in optical electronics such as OLED displays and improved drug delivery systems for treating cancer and other diseases. The particles can be directed to a body site via an antibody activated nanoparticles or nanoparticles activated with other agents to direct the nanoparticles to a given body site and irradiated them resulting in: (a) the release of site specific pharmaceuticals, (b) the thermal death of cells or foreign organisms in the body site, (c) the release of enzyme inhibitors or enzyme activators, (d) the release of antigens to invoke an immune response, (d) the absorption of bodily fluids, enzymes, proteins, poisons, metals, *etc.*, or (e) other processes.

[0018] The present invention relates to oxide, metal, or metal oxide core nanoparticles having formed on the surface thereof nanorods of noble metals or alloys, where the rods are grown on or from the surface assuming uniform and/or non-uniform directions and orientations on the surface providing for a different format for achieving light-to-heat energy transfer (*i.e.*, the plasmon resonance of rod-coated cores is distinct from that of nanoshells). These particles have optical properties ideally suited for electrooptical devices, drug delivery, and cell targeting to thermally kill cells at designated body sites.

[0019] The present invention relates to a drug-delivery system comprising a discrete noble metal or alloy shell nanoparticle or noble metal or alloy rod-bearing nanoparticle coated with a biocompatible hydrogel and to biocompatible hydrogel-coated nanoparticles impregnated with an effective amount of at least one pharmaceutically active compound, where the nanoparticles are prepared having a plasmon resonance in the near-IR spectral range (a tissue-transparent window). The plasmon resonance is tuned by controlling the diameter of the nanoparticle and the thickness of the shell or the dimensions of the rods deposited or formed on the particle's surface – for shells, larger diameters and smaller shell thicknesses shift the plasmon resonance to longer wavelengths or lower frequencies.

[0020] The present invention also relates to a method for preparing biocompatible hydrogel-coated noble metal or alloy shell nanoparticles or biocompatible hydrogel-coated noble metal or alloy rod-bearing nanoparticles including the steps of contacting shell nanoparticles or rod-bearing nanoparticles with a modifier including a noble metal reactive group or moiety and a hydrogel initiator group or moiety. During or after the shell nanoparticles or rod-bearing nanoparticles are reacted with the modifier, a hydrogel is grown on the noble metal shell nanoparticles or rod-bearing nanoparticles forming hydrogel-coated noble metal or alloy shell nanoparticles or rod-bearing

nanoparticles. Alternatively, the method includes the steps of contacting the noble metal or alloy shell nanoparticles or rod-bearing nanoparticles with a hydrogel-templating agent to form templated shell nanoparticles or rod-bearing nanoparticles, which are contacted with a hydrogel-forming solution to generate biocompatible hydrogel-coated noble metal or alloy shell nanoparticles or rod-bearing nanoparticles.

[0021] The present invention also relates to a method for preparing a drug-delivery system including the step of contacting a biocompatible hydrogel-coated noble metal or alloy shell nanoparticle or rod-bearing nanoparticle with an effective amount of a pharmaceutical compound under conditions to cause impregnation of the hydrogel coating with the pharmaceutical compound.

[0022] The present invention also relates to a method for delivering a pharmaceutical compound to a tissue site including the step of administering hydrogel-coated noble metal shell nanoparticles or rod-bearing nanoparticles impregnated with an effective amount of the pharmaceutical compound and then exposing the tissue site to light corresponding to the plasmon resonance of the nanoparticles causing the hydrogel to collapse releasing the pharmaceutical compound.

[0023] For applications involving the use of hydrogel-coated nanoparticles for thermally activated delivery of a given material or thermally activated absorption of a material, the nanoparticles are selected from the group of core particles having formed thereon a metallic shell having a plasmon resonance in a tissue-transparent frequency of electromagnetic light (*e.g.*, the near IR or others), core particles having formed thereon an alloy metallic shell having a plasmon resonance in a tissue-transparent frequency of electromagnetic light (*e.g.*, the near IR or others), core particles having formed thereon metallic nanorods or metallic alloy nanorods having a plasmon resonance in a tissue-transparent frequency of electromagnetic light (*e.g.*, the near-IR or others), metallic nanoparticles having formed thereon a metallic shell having a plasmon resonance in a tissue-transparent frequency of electromagnetic light (*e.g.*, the near-IR or others), metallic nanoparticles having formed thereon an alloy metallic shell having a plasmon resonance in a tissue-transparent frequency of electromagnetic light (*e.g.*, the near-IR or others), metallic nanoparticles having formed thereon metallic nanorods or metallic alloy nanorods having a plasmon resonance in a tissue-transparent frequency of electromagnetic light (*e.g.*, the near-IR or others), or other similar nanoparticles having a metallic coating having a plasmon resonance in a tissue-transparent electromagnetic frequency range.

## **DESCRIPTION OF THE DRAWINGS**

[0024] The invention can be better understood with reference to the following detailed description together with the appended illustrative drawings:

[0025] Figure 1 THPC gold-silver alloy seeds on silica particles. (a) UV-vis spectra of pure THPC alloy seeds and deposited alloy seeds on silica particles. (b) TEM image of alloy seeds on silica particles. (c) FE-SEM image of alloy seeds on silica particles.

[0026] Figure 2 EDX spectrum of alloy seeds deposited on silica particles

[0027] Figure 3 UV-vis spectra of alloy seed-gold shell particles

[0028] Figure 4 TEM images of THPC alloy seed-gold shell particles

[0029] Figure 5 UV-vis spectra of silver core-gold shell of various sizes and thicknesses of cores and shells. (a) 45 nm silver core with different thicknesses of gold shell. (b) 55 nm silver core thickness with different thicknesses of gold shell. (c) 75 nm silver core with different thicknesses of gold shell.

[0030] Figure 6 TEM images of silver core-gold shell particles

[0031] Figure 7 FE-SEM images of silver core- gold shell particles

[0032] Figure 8 UV-vis spectra of silica core-silver nanorod particles

[0033] Figure 9 FE-SEM images of silica core-silver nanorod particles. (a) Silver nanorod 71-5. (b) Silver nanorod 71-6.

[0034] Figure 10 TEM images of silica core-silver nanorod particles. (a) Silver nanorod 73-1. (b) Silver nanorod 73-6.

[0035] Figure 11 FE-SEM images of discrete hydrogel-coated gold nanoshell particles. (a) Discrete hydrogel-coated gold nanoshell (120 nm core) particles. (b) Discrete hydrogel-coated gold nanoshell (100 nm core) particles.

[0036] Figure 12 TEM images of discrete hydrogel-coated gold nanoshell particles. (a) Discrete hydrogel-coated gold nanoshell (120 nm core) particles. (b) Discrete hydrogel-coated gold nanoshell (100 nm core) particles.

[0037] Figure 13 Schematic illustrates a preferred discrete hydrogel coating process

[0038] Figure 14 Absorbance spectra of hydrogel-coated gold particles in (a) neutral and (b) acidic or basic media.

[0039] Figure 15 Illustrates FE-SEM images of (a) bare gold nanoparticles (~60 nm), (b) hydrogel-coated gold nanoparticles (~100 nm), (c) hydrogel-coated gold particles (~130 nm), and (d) hydrogel-coated gold nanoparticles (~230 nm).

[0040] Figure 16 EDX spectrum of hydrogel-coated gold nanoparticles.

[0041] Figure 17 A plot of particle size verse pH for bare gold nanoparticles and hydrogel-coated gold nanoparticles

[0042] Figure 18 A plot of particle size verses temperature for bare gold nanoparticles and hydrogel-coated gold nanoparticles

[0043] Figure 19 FE-SEM image of hydrogel-coated gold nanoshells (nanoshell core ~ 100 nm with a thin coating).

[0044] Figure 20 FE-SEM image of hydrogel-coated gold nanoshells (nanoshell core ~ 100 nm with a thick coating).

[0045] Figure 21 FE-SEM image of hydrogel-coated gold nanoshells (nanoshell core ~ 120 nm with a thin coating).

#### **DETAILED DESCRIPTION OF THE INVENTION**

[0046] The inventors have found that nanoparticles can be prepared from oxide, metal oxide, metals, or metal alloys having optionally thin metal or metal alloys shell grown, deposited or formed thereon to form nanoshells with oxide, metal oxide and/or metal cores, where the nanoparticles and nanoshells have a plasmon resonance so that they change temperature upon irradiation with light having a frequency or frequency range including the plasmon resonances of the nanoparticles and/or nanoshells. These nanoparticles and/or nanoshells, many of which are new, novel and unique, can also include a hydrogel or other polymeric structure formed thereon, deposited thereon, coated thereon, or polymerized thereon to form hydrogel or polymer coated nanoparticles or nanoshells. These hydrogel or polymer possess thermal properties that allow them to undergo a volume change or transition between a collapsed and an opened state. The transition can be used to deliver a material to: (a) a body site of an animal including a human, (b) a reaction medium, (c) an organic or inorganic matrix, (d) a solution, or (e) any other environment. The transition can also be used to absorb a material instead of delivering a material to a site (body or not), solution, reaction medium, a matrix, or any other environment.

[0047] The following examples are offered by way of illustration and are not intended to limit the invention in any manner.

#### **Silica core – Alloy Seed – Gold Shell**

##### **EXAMPLE I**

[0048] The present invention relates to improved metal oxide cores with noble metal shells, where

the shells are an alloy and have improved optical characteristics. The method for making the shells improves structure, size, and optical properties.

**[0049] Materials:** All chemicals were purchased from as indicated in parenthesis. Formaldehyde, sodium hydroxide, ammonium hydroxide (30%  $\text{NH}_3$ ), sodium borohydride, hydrochloric acid, and nitric acid (all from EM Science), potassium carbonate (J. T. Baker), hydrogen tetrachloroaurate-(III) hydrate (Au 99.9%, Strem), tetraethylorthosilicate, terakis(hydroxymethyl)phosphonium chloride, 3-aminopropyltrimethoxysilane (all from Aldrich), ethanol (McKormick Distilling Co.), silver nitrate (Mallinckrodt). All the chemicals were used as received without further purification. Highly pure water was purified to a resistance of 10  $\text{M}\Omega$  (Milli-Q Reagent Water System; Millipore Corporation) and filtered through 0.22  $\mu\text{m}$  filter to remove any aggregated impurities. All glassware were cleaned in an aquaregia (3:1,  $\text{HCl}:\text{HNO}_3$ ) solution first then cleaned in base bath (saturated KOH in isopropyl alcohol) and rinsed in Milli-Q water prior to use.

**[0050] Characterization Methods:** All products were examined by ultraviolet-visible (UV-vis) spectroscopy, field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS) and energy diffusive X-ray (EDX).

**[0051]** For the optical properties, the UV-vis spectra were obtained by using a Carry 50 Scan UV-visible spectroscopy over the range from 300 to 1100 nm wavelength. All samples were centrifuged and redispersed and diluted in Milli-Q water and transferred into a quartz cell with optical glass windows.

**[0052]** For the morphology and distribution of silica core gold shell nanoparticles, FE-SEM was performed using a JSM 6330F (JEOL) instrument operating at 15 kV and TEM was carried out using a JEM-2000 FX electron microscope (JEOL) at accelerating voltage 200 kV equipped with EDX (Link analytical EXL, Oxford) analyzer. In order to get better images from FE-SEM, the core-shell particles were deposited on the gold-coated silicon wafer and completely dried prior to coating with carbon. The samples were then coated with carbon film to improve electrical conductivity. For the TEM analysis, the samples were deposited on 300 mesh Holey carbon-coated copper grid and dried before they were examined.

**[0053]** In order to measure the particle diameters, DLS was performed using ALV-5000 Multiple Tau Digital Correlation instrument operating at a light source 514.5 nm wavelength and a fixed scattering angle of 90°. The sample diameters were compared to the data from TEM and FE-SEM for the consistency.

**[0054] Preparation of amine-functionalized silica nanoparticles:** This is a modification of the well-known Stöber method. (Stober, W.; Fink, A.; Bohn, E. *J. Colloid Interface Sci.* **1968**, 26, 62.) Ammonium hydroxide (13.4 mL) was mixed with 100 mL of absolute ethanol in a 500 mL two-necked round bottom flask. The mixture was stirred for 15 minutes and TEOS was quickly added. The particle formation was observed in 30 minutes due to the color change of the mixture from colorless to milky white. From the FE-SEM and TEM images as well as DLS data, the particle diameters were ~350 nm spherical shape overall (data not shown). APTMS (0.16 mL) was then added to previously prepared 100 mL of silica particles in a 250 mL two-necked round bottom flask with stirring. The mixture was vigorously stirred for 24 hours at room temperature and 10 mL of ethanol was added drop-wise during the reflux step to enhance covalent bonding of APTMS onto the silica particles, while it was heated to 85°C for 1 hour. (Waddell, T. G.; Leyden, D. E.; DeBello, M. T. *J. Am. Chem. Soc.* **1981**, 103, 5303. van Blaaderen, A.; Vrij, A. J. *J. Colloid Interface Sci.* **1993**, 156, 1.) The solution was centrifuged at 3000 rpm (revolution per minute) for 1 hour and redispersed in 100 mL of ethanol twice. There were no considerable differences between the unfunctionalized silica particles and functionalized silica particles from the FE-SEM, TEM, as well as DLS results.

**[0055] THPC gold-silver alloy seed preparation:** Gold-silver alloy nanoparticles were prepared using THPC (THPC gold-silver alloy) via modification of the Duff et al. method. (Duff, D. G.; Baiker, A. *Langmuir* **1993**, 9, 2301. Duff, D. G.; Baiker, A. *Langmuir* **1993**, 9, 2310.) One (1) mL of sodium hydroxide (1 mol) and 2 mL of THPC solution (12 µL of 80% THPC in 1 mL of water) was mixed with 100 mL of Milli-Q water in a 250 mL flask. The reaction mixture was vigorously stirred for 5 minutes, after that 2 mL of 1% aqueous AgNO<sub>3</sub> and 1% HAuCl<sub>4</sub>·3H<sub>2</sub>O were added quickly to the mixture. The mixture solution was stirred about 30 more minutes. The color of the solution changed very quickly from colorless to dark reddish yellow indicating the formation of gold-silver alloy particles ~4-6 nm in diameter. This solution was stored in the refrigerator for three days prior to use.

### **Silver Core – Gold Shell**

#### **EXAMPLE II**

**[0056]** The present invention relates to metallic nanoparticles such as silver nanoparticles having deposited thereon a shell of a noble metal such as gold. These metal-core-noble-metal-shell particles have improved optical properties for use in optical electronics such as OLED displays and improved

drug delivery systems for the site specific delivery of drugs for cancer treatments or other diseases where the particles can be directed to a body site and irradiated resulting in thermal death of cells in the body site or delivery of drugs to treat symptoms or ameliorate symptoms of diseases. For non *in vivo* use the particles can be used for light induced release or absorption of a desired material.

**[0057] Materials:** Sodium citrate dihydrate, nitric acid, hydrochloric acid (EM Science), potassium carbonate (J. T. Baker), hydrogen tetrachloroaurate-(III) hydrate (Strem), silver nitrate (Mallinckrodt) were purchased from indicated companies. All the chemicals were used as received without purification. Water was purified to a resistance of 18 M $\Omega$  (Academic Milli-Q Water System; Millipore Corporation) and filtered using 0.22  $\mu$ m filter. All glassware used in the experiment were cleaned in an aquaregia solution (3:1, HCl:HNO<sub>3</sub>) first and cleaned in base bath (saturated KOH in isopropyl alcohol) prior to use.

**[0058] Characterization Methods:** All the nanoparticles were characterized by ultraviolet-visible (UV-vis) spectroscopy for the optical properties, by field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM) for the morphology, by dynamic light scattering (DLS) for the diameters of nanoparticles, by energy diffusive X-ray (EDX) for elemental compositions.

**[0059]** First, JSM 6330F (JEOL) FE-SEM and JEM-2000 FX electron microscope (JEOL) TEM was used to observe the morphology and particle distribution of the particles. FE-SEM was operated at accelerating voltage 15 kV and equipped with a setup for elemental analysis by EDX (Link ISIS software series 300, Oxford Instruments) and TEM was accomplished at accelerating voltage 200 kV. The samples were placed on Formvar-coated copper grid and dried at room temperature overnight before the FE-SEM and TEM analysis. The sample for FE-SEM was then coated with carbon sputtering machine in order to get high-resolution images. The samples were examined by FE-SEM images (magnification 20,000-150,000X) and TEM images (100,000–500,000X) to show the morphology and overall uniformity of particles on the surface.

**[0060]** ALV-5000 Multiple Tau Digital Correlation instrument operating at a light source 514.5 nm wavelength and a fixed scattering angle of 90°C was used to measure nanoparticle sizes for the DLS measurements. The sample sizes were compared to the data from TEM and FE-SEM for the consistency.

**[0061]** A Cary 50 Scan UV-visible spectrometer was used over the range from 300-1100 nm wavelength to observe optical properties of nanoparticles. All samples were centrifuged and

redispersed in Milli-Q water to adjust concentration of each samples and transferred into a UV cell to measure the optical properties.

**[0062] Preparation of silver nanoparticle cores:** This is a modification of the well known Lee and Meisel method to make variable sizes of silver nanoparticles. (*Langmuir* 2001, 17, 574-577. *Journal of Colloid and Interface and Science*, 1983, 93, 545-555; *J. Phys. Chem.* 1982, 86, 3991.) 200 mL of a  $10^{-3}$  M  $\text{AgNO}_3$  solution was heated to boiling, and added 4 mL of a 1 % trisodium citrate as soon as it reaches boiling. The mixture was kept stirring and boiling for 45 minutes to get homogeneous silver nanoparticles. Other sizes of silver nanoparticles were prepared from different concentrations of silver nitrate with constant amount of sodium citrate.

**[0063]  $\text{K}_2\text{CO}_3$ -gold (K-gold) preparation:** To make K-gold solution, 0.05 g of potassium carbonate ( $\text{K}_2\text{CO}_3$ ) in 200 mL Milli-Q water was stirred for at least 15 min to dissolve  $\text{K}_2\text{CO}_3$  completely and added 4 mL of 1 wt%  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ . The color of solution changes from yellow to almost colorless within 40 min.

**[0064] Gold-coated silver nanoparticles by self-assembly:** In order to grow the gold layer on the silver nanoparticle cores, the prepared K-gold solution 8 mL was placed in a 25 mL beaker with stir bar and added prepared silver nanoparticle cores (0.5 to 6 mL) to produce different thickness of gold layers. The mixture was kept stirring at least 10 minutes and changed colors from light yellow to greenish blue. The mixture was left for at least one day to get complete coating and centrifuged at 2500 rpm (revolution per minute) for 1 hour using RC-3B Refrigerated Centrifuge (Sorvall Instruments) and redispersed in Milli-Q water for the analysis.

### Silica Core – Silver Nanorods

#### **EXAMPLE III**

**[0065]** The present invention relates to metal oxide core nanoparticles having formed on the surface of nanorods of noble metals, where the rods are grown from the surface assuming a variety of different direction and orientations on the surface. The nano-ceramic-core-noble-metal-nanorod particles have optical properties ideally suited for electrooptical devices, drug delivery, and cell targeting to thermally kill cells at targeted body sites.

**[0066] Materials:** The sodium hydroxide, ammonium hydroxide (30%  $\text{NH}_3$ ), trisodium citrate dihydrate, nitric acid, borohydride, hydrochloric acid from EM Science, 3-aminopropyltrimethoxysilane (APTMS), tetraethylorthosilicate (TEOS) from Aldrich, ethanol from McKormick Distilling Co., silver nitrate from Mallinckrodt, cetyltrimethyl ammonium bromide



(CTAB, 99+%) from Acros, and ascorbic acid from Chemalog were purchased from indicated companies. All the chemicals were used as received without purification. Water used in all reaction was purified to a resistance of 18 M $\Omega$  (Academic Milli-Q Water System; Millipore Corporation) and filtered using 0.22  $\mu$ m filter membrane. All glassware used in the experiment were cleaned in an aquaregia solution first and cleaned in base bath prior to use.

**[0067] Preparation of amine-functionalized silica nanoparticles:** This is a modification of the well-known Stöber method for making large silica nanoparticles. (Stober, W.; Fink, A.; Bohn, E. J. *Colloid Interface Sci.* **1968**, 26, 62) 26.8 mL of ammonium hydroxide was added to 200 mL of absolute ethanol in a 500 mL two-necked round bottom flask and was stirred for 30 min at 30°C. Tetraethylorthosilicate (TEOS) 6 mL was quickly added into the mixture at 30°C. The color change of the mixture from colorless to milky white was observed in about 30 minutes and kept stirring it for overnight. 0.5 mL of excess APTMS was then added to the solution. The mixture was vigorously stirred for another 6-8 hours and heated to 85°C for 1 hour to enhance covalent bonding of APTMS onto the silica particles. (Waddell, T. G.; Leyden, D. E.; DeBello, M. T. *J. Am. Chem. Soc.* **1981**, 103, 5303. van Blaaderen, A.; Vrij, A. J. *J. Colloid Interface Sci.* **1993**, 156, 1) The amine-functionalized silica particles were centrifuged RC-3B Refrigerated Centrifuge (Sorvall Instruments) at 2500 rpm (revolution per minute) for 1 hour and redispersed in 200 mL of ethanol twice. FE-SEM and TEM results showed no major differences between the unfunctionalized silica particles and functionalized silica particles from our experiment.

**[0068] The preparation of silver seeds attached to silica nanoparticles:** Silver seed (~3-4 nm in diameter) solution was prepared by an adaptation of the Nikhil et al. method to attach onto silica particles. [Nikhil R. Jana, Latha Gearheart and Catherine J. Murphy *Chem. Commun.* **2001**, 617. Nikhil R. Jana, Latha Gearheart, Catherine J. Murphy *Adv. Mater.* **2001**, 13, 1389.] A 100 mL aqueous solution containing each 0.25 mM AgNO<sub>3</sub> and trisodium citrate was stirred for 5 minutes and 2.4 mL of a 0.01 M borohydride solution was quickly added into the mixture. The color of solution changed from colorless to bright yellow in few seconds which indicates the formation of ~ 4 nm silver nanoparticles. After 1 hour later, the seed solution was mixed with 2 mL of amine-functionalized silica nanoparticles and stood for 2 hr at room temperature to make silver seed attached silicana nanoparticles by self-assembly. The mixture was then centrifuged at 3000 rpm for 1 hour, and the dark black-colored precipitate was redispersed in 100 mL of water. The mixture was sonicated for 5 minutes then centrifuged again for 30 minutes. The solution showed very light

yellow color after the precipitate was re-dispersed in 100 mL of water.

**[0069] The preparation of silver-rods grown on silica nanoparticles:** First, 0.5 mL of 10 mM AgNO<sub>3</sub> solution was mixed with 20 mL of 80 mM CTAB and mix them carefully. The 1 mL of 100 mM ascorbic acid and varying amount of silver seed attached silica nanoparticles (0.125 ~ 2 mL) were added to the mixture and gently stirred it for 5 minutes. 0.2 mL of 1 M NaOH was added at the last step and gently shaken for another 5 minutes. The solution showed color change in 10 minutes dependant on the amount of silver seed attached silica nanoparticles which that can control the size of silver nanorod onto silica surfaces. The final solution was centrifuge at 3000 rpm for 30 min to separate unreacted silver seed or free silver nanorod from the mixture. The precipitate was re-dispersed in 10mL of water and sonicated for 5 minutes and centrifuged again for 30 minutes. The solution showed yellow, red, brown, blue, or green colors dependant on the size of silver rods after the precipitate was re-dispersed in 100 mL of water.

#### **Synthesis of Hydrogel-Coated Gold Nanoparticles**

##### **EXAMPLE IV**

**[0070]** The present invention relates to a targeted drug-delivery or absorbing system including metal or alloy nanoparticles having deposited or grown thereon a hydrogel coating. The present invention also relates to hydrogel-coated nanoparticles impregnated with one or more pharmaceuticals or bioactive agents. The present invention also relates to a method for treating body sites by locating the impregnated hydrogel-coated nanoshell nanoparticles and irradiating the nanoparticles to release the pharmaceuticals or bioactive agents.

**[0071] Materials.** The monomer N-isopropylacrylamide (NIPAM) was obtained from Acros (99%), recrystallized in hexane, and dried under vacuum before use. N,N'-methylenebisacrylamide (BIS, Acros), Acrylic acid (AAc, Acros, 99.5%), potassium hydroxide (KOH, EM, 85%), nitric acid (HNO<sub>3</sub>, EM, 70%), ammonium persulfate (APS, EM, 98%), and oleic acid (OA, J. T. Baker) were all used as received from the indicated suppliers. Water used in all reactions, solution preparations, and polymer isolations was purified to a resistance of 10 MΩ (Milli-Q Reagent Water System, Millipore Corporation) and filtered through a 0.2 μm filter to remove any particulate matter. In the preparation of gold nanoparticles, trisodium citrate (EM, 99%) and hydrogen tetrachloroaurate (Strem, Au 99.9%) were used without purification.

**[0072] Preparation of Gold Nanoparticles.** Gold nanoparticles were prepared via the common technique of citrate reduction, which has been described in detail. (Frens, G. *Nature Phy. Sci.* 1973,

241, 20. Turkevich, J.; Stevenson, P. C.; Hillier, J. *Discussions Fara. Soc.* **1951**, *58*, 55. Goodman, S. L.; Hodges, G. H.; Trejdosiewicz, L. K.; Linvinton, D. C. *J. of Microscopy* **1981**, *123*, 201.) The sizes of our gold nanoparticles were always between 55 and 65 nm as judged by dynamic light scattering (DLS) and field emission scanning electron microscopy (FE-SEM). The glassware was cleaned first with strong acid (3/1 HCl/HNO<sub>3</sub>) and then with strong base (saturated KOH in isopropyl alcohol) before use.

**[0073] Synthesis of Hydrogel-Coated Gold Nanoparticles.** (Quanroni, L.; Chumanov, G. *J. Am. Chem. Soc.* **1999**, *121*, 10642. Clark, H. A.; Campagnok, P. J.; Wuskell, J. P.; Lewis, A.; Loew, L. *M. J. Am. Chem. Soc.* **2000**, *122*, 10234.) The hydrogel-coated gold nanoparticles were prepared by surfactant-free emulsion polymerization (SFEP) in aqueous solution. In a three-necked round-bottomed flask equipped with a reflux condenser and an inlet for argon gas, gold colloidal solutions were diluted with purified Milli-Q water to give a maximum of ~0.25 a.u. at 530 nm. The solution was purged with argon for 1 h and was bubbled through the solution for the duration of the reaction to remove any oxygen, which can intercept radicals and disrupt the polymerization. The solution was agitated using a football-shaped Teflon-coated magnetic stirring bar. Degassed oleic acid 1.6 mL (0.001 M), which has a low affinity toward gold, was then added to the stock solution under argon. The mixture was stirred for 1 h and placed in an ultrasonicator for 15 minutes. An approximately 94:6 wt% ratio of NIPAM 26.1 mL (0.01 M):AAc 1.6 mL (0.01 M) and 2 mL of BIS (0.01 M) was then added and stirred for 15 minutes to give homogeneity. The solution was then heated to 71°C in an oil bath, and then APS 0.8 mL (0.01 M) was added to initiate the polymerization. The reaction time, which depended on the amount of starting materials, was varied between 6 and 8 h. At the end of this period, the solution was cooled and filtered through a 1 µm membrane to remove any micron-sized impurities and/or any aggregated particles. The filtered solution was centrifuged at 20°C for 2 h at 3500 rpm with RC-3B Refrigerated Centrifuge (Sorvall Instruments), and the supernatant was separated to remove unreacted materials, soluble side products, and seeds of pure polymer. The purified nanoparticles were then diluted with pure Milli-Q water and stored at room temperature for later use. The size of the hydrogel-coated gold particles was varied between 100 and 230 nm by controlling the amount of monomer and initiator as well as the reaction time.

**[0074] Characterization of Gold and Hydrogel-Coated Gold Nanoparticles.** To characterize the pure gold nanoparticles and hydrogel-coated gold nanoparticles, we used field emission scanning

electron microscopy (FE-SEM), energy diffusive X-ray (EDX) analysis, ultraviolet-visible (UV-vis) spectroscopy, and dynamic light scattering (DLS). Due to our interest in thick hydrogel coatings, our most thorough analyses were focused on the hydrogel-coated gold nanoparticles having ~230 nm diameters.

[0075] We employed a Cary 50 Scan UV-vis optical spectrometer (Varian) with Cary Win UV software to characterize the optical properties of the bare gold nanoparticles and the hydrogel-coated gold nanoparticles. UV-vis spectra of the prepared gold nanoparticles were collected by diluting the particles with Milli-Q water, transferring them to an optical glass cell, and scanning over a range of wavelengths (400-1100 nm). The hydrogel-coated gold nanoparticles were analyzed as prepared (i.e., without dilution). For consistency, UV-vis spectra of the distinct batches of nanoparticles were collected both before and after coating with the hydrogel.

[0076] Analysis by FE-SEM was performed using a JSM 6330F (JEOL) instrument operating at 15 kV and equipped with a setup for elemental analysis by EDX (Link ISIS software series 300, Oxford Instruments). To collect both FE-SEM images and EDX data, the gold nanoparticles and hydrogel-coated nanoparticles were deposited on Formvar-coated copper grids and completely dried at room temperature overnight prior to analysis. The samples were then coated with a carbon film (2.5 nm thick) using a vacuum sputterer. The gold and hydrogel-coated gold nanoparticles were examined by FE-SEM (magnification 20,000-100,000X) to demonstrate the overall morphological uniformity of the particles and by EDX to confirm the presence of the gold nanoparticle core.

[0077] For the DLS measurements, an ALV-5000 Multiple Tau Digital Correlation instrument operating at a light source wavelength of 514.5 nm and a fixed scattering angle of 90°C was used to measure particle size as a function of temperature and pH for gold and hydrogel-coated gold nanoparticles. The samples were measured at dilute concentrations with precise control over the temperature (especially at higher temperatures to reduce artifacts resulting from convection currents in the samples). For all samples, data were collected from 20–60°C. All data showed good Gaussian distribution curves, and the standard deviation of the distribution was 5 to 20 % of the mean for all samples.

### **Synthesis of Discrete Hydrogel-Coated Gold Shell Nanoparticles**

#### **EXAMPLE V**

[0078] The present invention relates to a targeted drug-delivery or absorbing system including nanoshell nanoparticles having deposited or grown thereon a hydrogel coating. The present

invention also relates to hydrogel-coated nanoshell nanoparticles impregnated with one or more pharmaceuticals or bioactive agents. The present invention also relates to a method for treating body sites by locating the impregnated hydrogel-coated nanoshell nanoparticles and irradiating the nanoparticles to release the pharmaceuticals or bioactive agents.

**[0079] Materials:** The N-isopropylacrylamide (NIPAM) monomer was obtained from Acros (99%), recrystallized in hexane, and dried at room temperature before use. Other chemicals were used: acrylic acid (AAc), N,N'-methylenebisacrylamide (BIS) from Acros and nitric acid, hydrochloric acid, ammonium persulfate (APS), sodium hydroxide, ammonium hydroxide (30% NH<sub>3</sub>), formaldehyde, potassium carbonate, trisodium citrate from EM Science, terakis(hydroxymethyl)phosphonium chloride (THPC), 3-aminopropyltrimethoxysilane tetraethylorthosilicate (APTMS) from Aldrich, hydrogen tetrachloroaurate (Au 99.9%) from Strem, and ethanol from McKormick Distilling Co., oleic acid from J. T. Baker. Water used in all reactions, solution purifications, and polymer isolations was used as a resistance of 18 MΩ (Academic Milli-Q Water System, Millipore Corporation) and filtered through a 0.22 μm filter. All glassware used in the experiment were cleaned in an aqua regia solution first then cleaned in base bath and rinsed in Mill-Q water prior to use.

**[0080] Characterization Methods:** All the particles were analyzed by ultraviolet-visible (UV-vis) spectroscopy, field emission scanning electron microscopy (FE-SEM), dynamic light scattering (DLS), transmission electron microscopy (TEM) and energy diffusive X-ray (EDX).

**[0081]** JSM 6330F (JEOL) FE-SEM instrument was used to observe the morphology of the particles operating at 15 kV. In order to get high resolution of the images the gold seed deposited silica nanoparticles were placed on the carbon-coated copper grid and completely dried at room temperature overnight prior to the carbon coating. The samples were then coated with carbon films using sputtering equipment to improve electrical conductivity. The samples were examined by FE-SEM images (magnification 20,000-150,000X) to show the overall uniformity and morphology of particles on the surface.

**[0082]** A JEM-2000 FX electron microscope (JEOL) TEM analysis was accomplished at accelerating voltage 200 kV. All the TEM samples were deposited on 300 mesh Holey carbon coated copper grids and dried before they were examined.

**[0083]** The UV-vis spectra were obtained by using a Cary 50 Scan UV-visible spectroscopy over the range from 300-1100 nm wavelength. All samples were centrifuged and redispersed in Milli-Q

water and transferred into a UV cell with optical glass windows.

**[0084] Preparation of amine-functionalized silica nanoparticles:** This is a modification of well-known Stöber method for making large silica nanoparticles. (Stober, W.; Fink, A.; Bohn, E. J. *Colloid Interface Sci.* 1968, 26, 62.) 26.8 mL of ammonium hydroxide was added to 200 mL of absolute ethanol in a 500 mL two-necked round bottom flask and stirred for 30 minutes at 30 °C. Six (6) mL of TEOS was quickly added into the mixture, and the silica particle formation was observed by the color changes of the solution from colorless to milky white within 30 minutes. The mixture was kept stirring for 24 hour and 0.5 mL of excess APTMS was then added to the solution. The mixture was vigorously stirred for another 6-8 hours and heated to 85°C for 1 hour to enhance covalent bonding of APTMS onto the silica particles. (Waddell, T. G.; Leyden, D. E.; DeBello, M. T. *J. Am. Chem. Soc.* 1981, 103, 5303. van Blaaderen, A.; Vrij, A. J. *Colloid Interface Sci.* 1993, 156, 1.) The solution was centrifuged at 2500 rpm (revolution per minute) for 1 h using RC-3B Refrigerated Centrifuge (Sorvall Instruments) and redispersed in 200 mL of ethanol twice. FE-SEM and TEM results showed no major differences between the unfunctionalized silica particles and functionalized silica particles from our experiments (data now shown).

**[0085] The preparation THPC gold seed and K-gold solution:** The THPC gold seed solution was made using a modification of the Duff et al method. (Duff, D. G.; Baiker, A. *Langmuir* 1993, 9, 2301. Duff, D. G.; Baiker, A. *Langmuir* 1993, 9, 2310. Teo, B. K.; Keating, K.; Kao, Y-H. *J. Am. Chem. Soc.* 1987, 109, 3494.) To make ~2-4 nm THPC gold seed nanoparticles, 1 mL of 1 M NaOH, 2 mL of THPC (12 µL of 80% THPC in 1 mL of water), and 200 mL of Milli-Q water were mixed in a 250 mL flask and vigorously stirred at least 15 minutes. 4 mL of 1% aqueous  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  were added quickly to the solution and stirred about 30 more minutes. The color of the solution changed very quickly from colorless to dark red. The size of gold nanoparticles could be varied, but our particles possessed diameters of ~2-3 nm. This solution was then stored in the refrigerator for at least three days.

**[0086]** For the K-gold solution, 0.05 g potassium carbonate in 200 mL water was stirred for at least 15 minutes to ensure dissolving of  $\text{K}_2\text{CO}_3$  and then 2 mL of 1 %  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  was added. The color of the solution changed from yellow to colorless within 40 minutes.

**[0087] The formation of THPC gold nanoparticles attached to silica nanoparticles:** This is the modification of Westcott et. al. method to make gold seed attached onto silica core particles. (Westcott, S. L.; Oldenburg, S. J.; Lee, T. R.; Halas, N. J. *Langmuir* 1998, 14, 5396.) The THPC

gold nanoparticles were deposited onto the silica particles by mixing THPC gold nanoparticles and amine-functionalized silica nanoparticles for overnight. About 1 mL of amine-functionalized silica particles dispersed in ethanol was placed into a 50 mL of three-day aged THPC gold nanoparticles in a centrifuge tube. The mixture was shaken for a couple of minutes and left overnight to attach gold seeds to silica particles by self-assembly. The mixture was then centrifuged at 3000 rpm for 1 hour and the dark red-colored precipitate were redispersed in 50 mL of water. The mixture was briefly sonicated for 5 minutes and centrifuged again for additional 60 minutes. The solution showed very light red color after the precipitate was redispersed in 50 mL of water.

**[0088] The gold nanoshell growth:** (T. Pham; J.B. Jackson; N.J. Halas; T.R. Lee *Langmuir* 2002, 18, 4915.) In order to make complete gold layer onto THPC gold seed attached silica nanoparticles, the prepared K-gold solution (4 mL) was placed in a 25 mL beaker and added different amount of THPC gold seed attached silica nanoparticles (0.1 to 2 mL) to produce different thickness of gold shells. The mixture was stirred at least 5 minutes and added 0.01 mL of reducing agents such as formaldehyde and borohydride to reduce K-gold solution. The color change of solution took place from colorless to blue, green, and yellowish green dependent on the shell thickness. The gold nanoshells were centrifuged and redispersed in Milli-Q water to remove unreacted free gold seed particles.

**[0089] Preparation of hydrogel-coated gold nanoshell particles.** The hydrogel-coated gold nanoshell particles were prepared by modification of the method from Quanroni et. al. (Quanroni, L.; Chumanov, G. *J. Am. Chem. Soc.* 1999, 121, 10642.) The gold shell solution was diluted with 0.001M potassium carbonate solution to slow down the aggregation phenomenon and to adjust the concentration of solution, which has a absorption maximum of ~0.6 a.u. at 800 nm using UV-vis spectroscopy.

**[0090]** The hydrogel-coated gold nanoshell particles were prepared in a 500 mL three-necked round-bottomed flask equipped with a reflux condenser and filled with argon gas. Oleic acid (0.00174 mL;  $3 \times 10^{-5}$  mol) was added to the gold shell solution and stirred for 45 minutes and then placed in an ultrasonic bath for 15 minutes. An approximately 94:6 wt% ratio of NIPAM (0.125 g;  $4.4 \times 10^{-3}$  mol): AAc (0.0075 g;  $5 \times 10^{-4}$  mol) and cross-linker BIS (0.003 g;  $1 \times 10^{-4}$  mol) were then added, and the mixture was stirred for 15 minutes. The solution was heated to 70°C in an oil bath, and then air-free APS (0.0048 g;  $1 \times 10^{-4}$  mol) was quickly added to initiate the polymerization. The reaction was allowed to proceed for ~16 hours, after that the solution was filtered through a 1  $\mu$ m membrane

to remove any micron-sized impurities. The filtered solution was centrifuged at 2500 rpm for 1 hour at 30°C with an RC-3B Refrigerated Centrifuge (Sorvall Instruments). Afterward, the top layer containing unreacted materials or water-soluble side products was removed by decantation. The purified hydrogel-coated gold nanoshell particles were then diluted with Milli-Q water and stored at room temperature for subsequent analyses.

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[0091] The following references are have been cited in the specification above:

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20. Clark, H. A.; Campagnok, P. J.; Wuskell, J. P.; Lewis, A.; Loew, L. M. *J. Am. Chem. Soc.* 2000, 122, 10234.



## **CLAIMS**

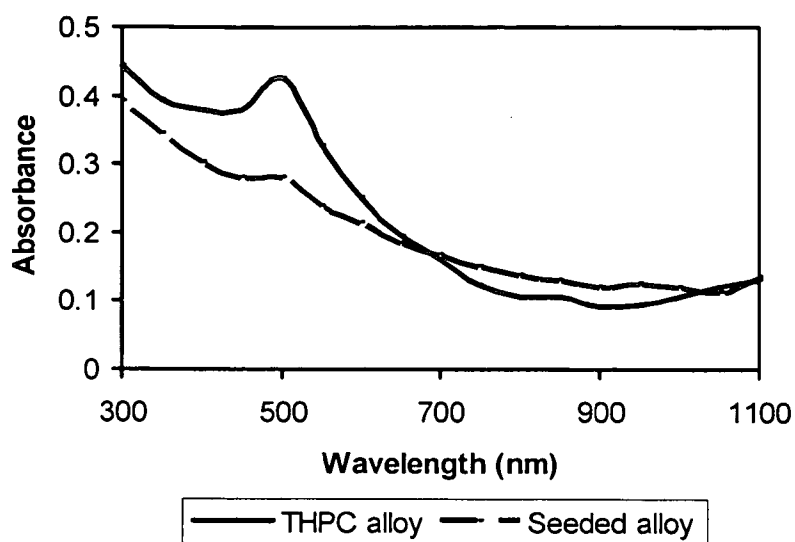
**[0081]** All inventions substantially as described herein.

## **ABSTRACT**

Nanostructures are disclosed that are ideally suited for microelectronics, medical treatment, drug delivery systems, targeted thermal absorption media, or other similar applications, where the nanoparticles include metal oxide nanoparticles and metallic nanoparticles including a metallic nanoshell or metallic nanorods deposited on the surface of the particles having a plasmon resonance. For *in vivo* medical applications, the plasmon resonance is tuned to a tissue-transparent frequency range. Hydrogel-coated nanostructures are also disclosed, which are capable of transitioning between a collapsed hydrogel and an opened hydrogel via thermal activation induced by electromagnetic irradiation.

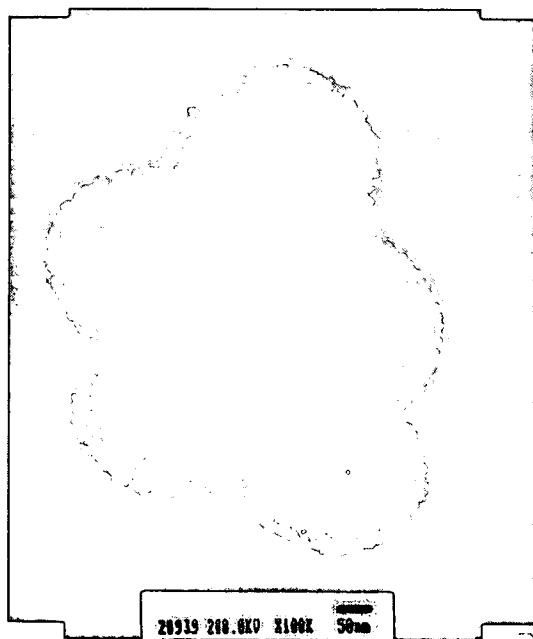
## FIGURES

Figure 1

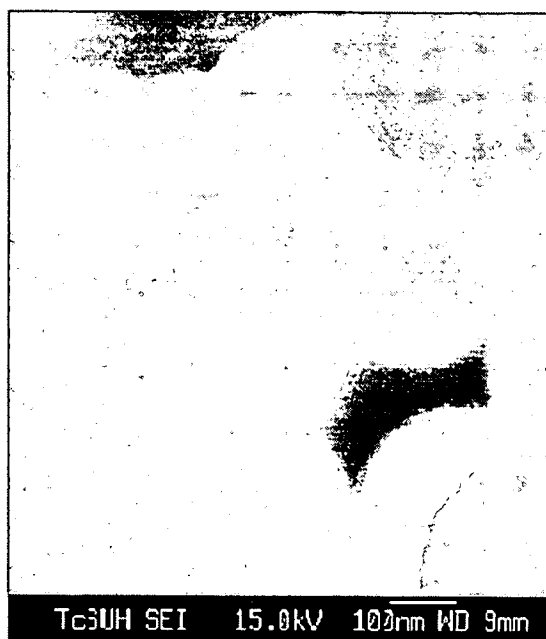


(a)

THPC gold-silver alloy seeds on silica particles. (a) UV-vis spectra of pure THPC alloy seeds and deposited alloy seeds on silica particles.

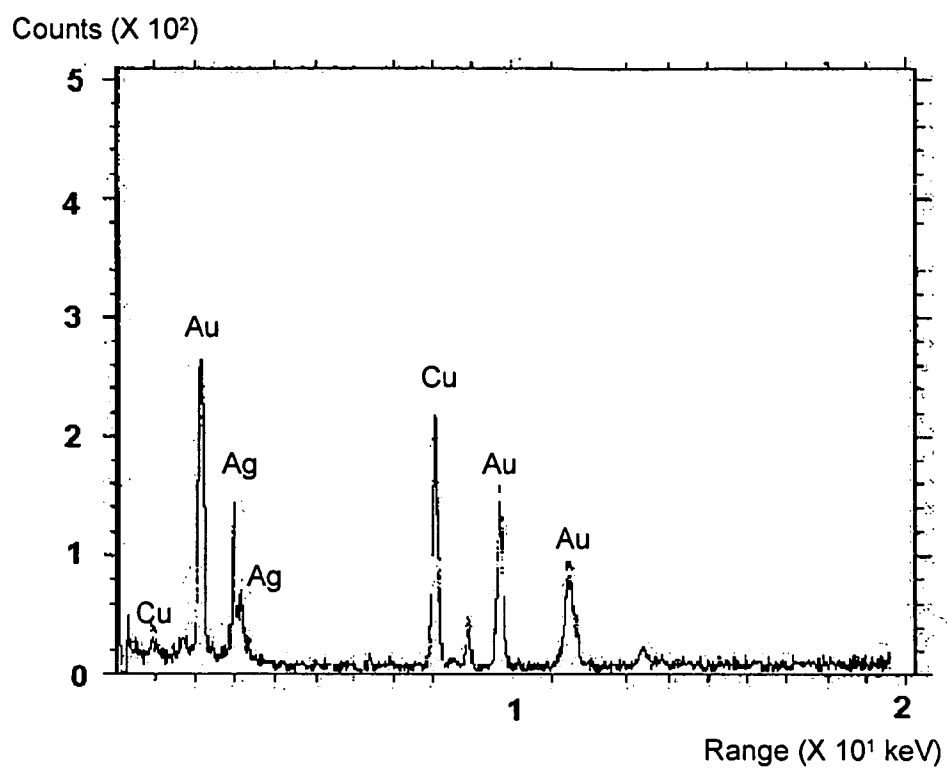


(b)

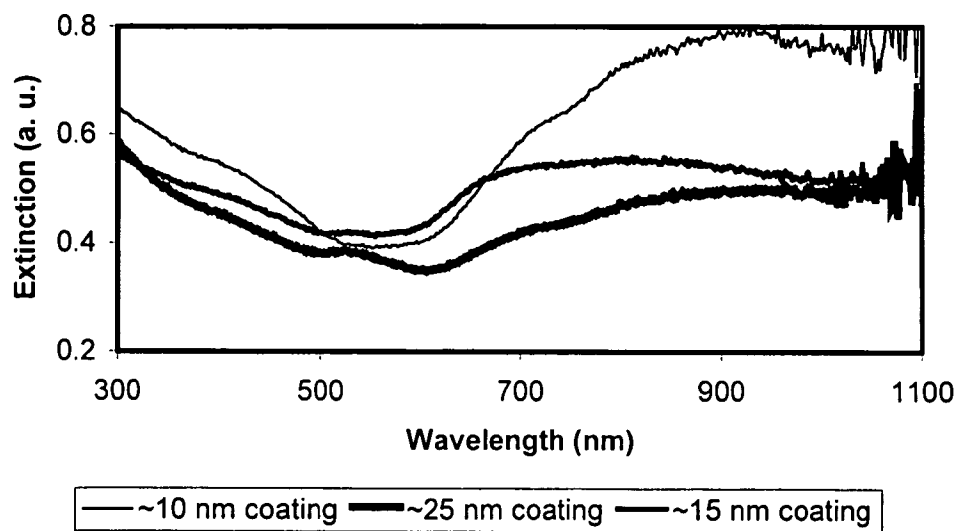


(c)

(b) TEM image of alloy seeds on silica particles. (c) FE-SEM image of alloy seeds on silica particles.

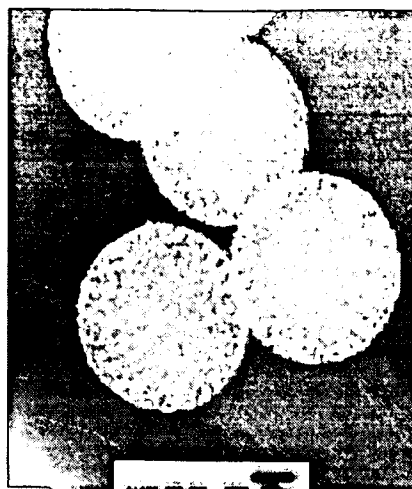
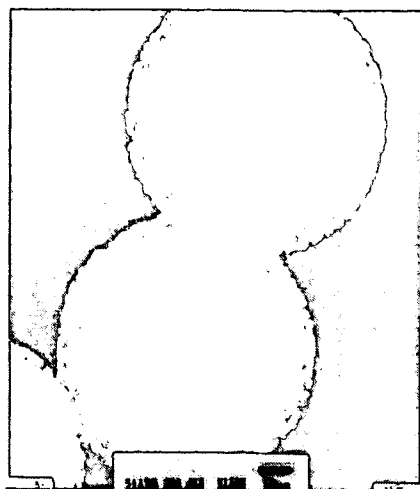


**Figure 2** EDX spectrum of alloy seeds deposited on silica particles.

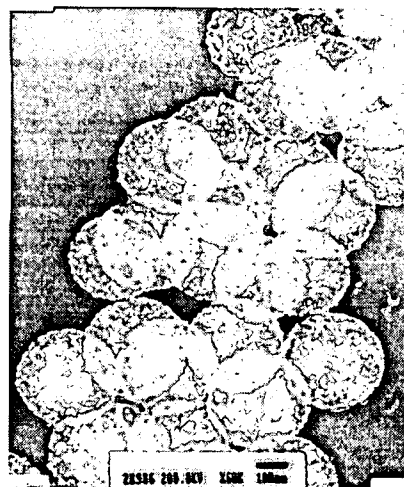
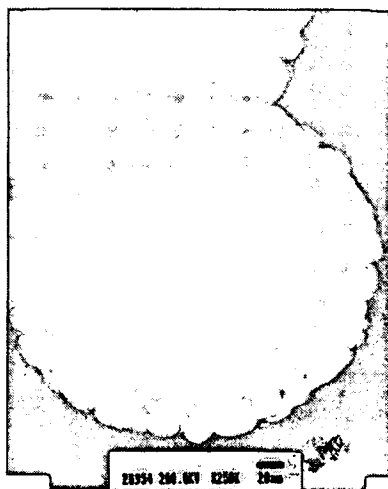


**Figure 3** UV-vis spectra of alloy seed-gold shell particles.

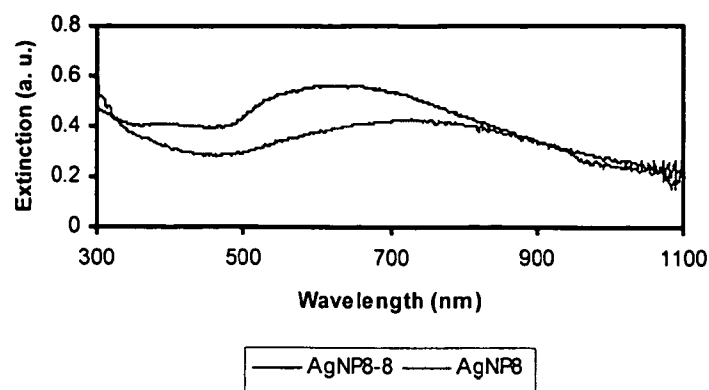
~ 10 nm shell coating



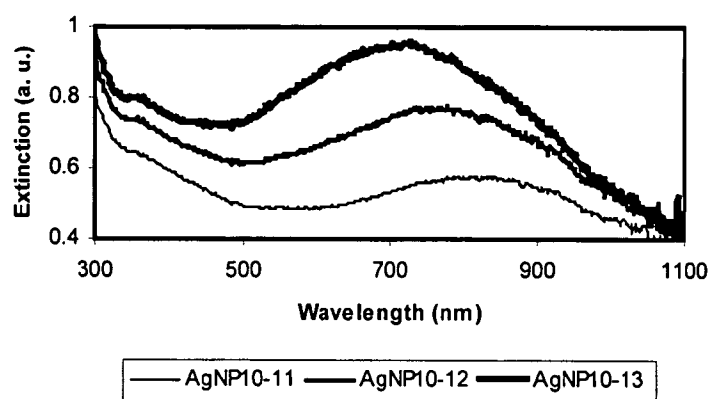
~25 nm shell coating



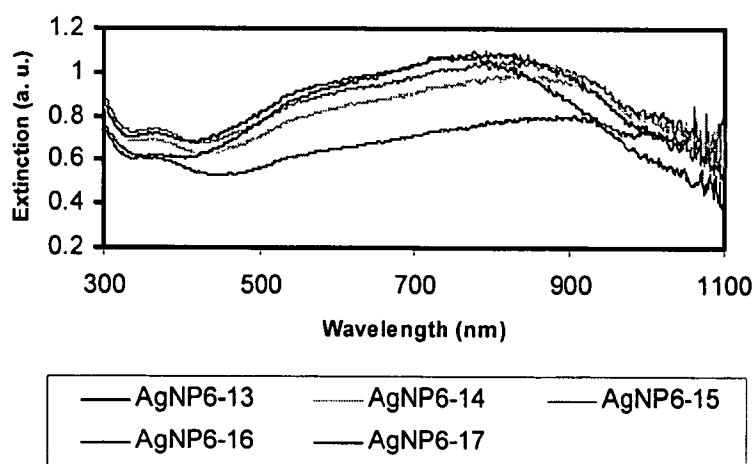
**Figure 4** TEM images of THPC alloy seed-gold shell particles.



(a) 45 nm silver core with different thickness of gold shell

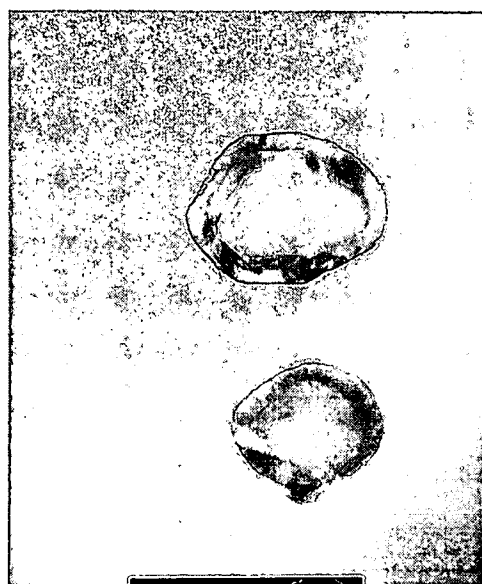


(b) 55 nm silver core thickness with different thicknesses of gold shell

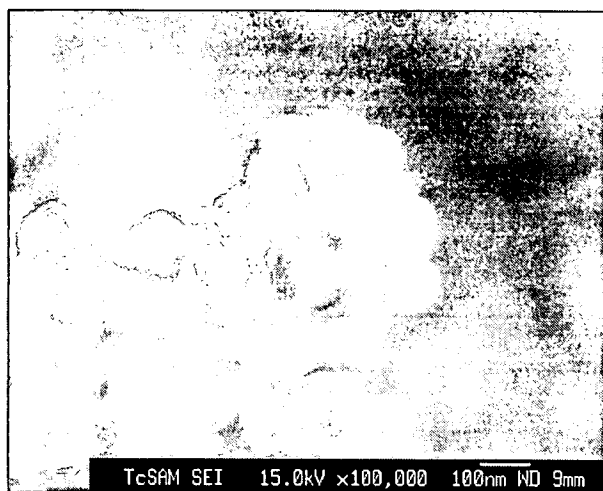


(c) 75 nm silver core with different thickness of gold shell

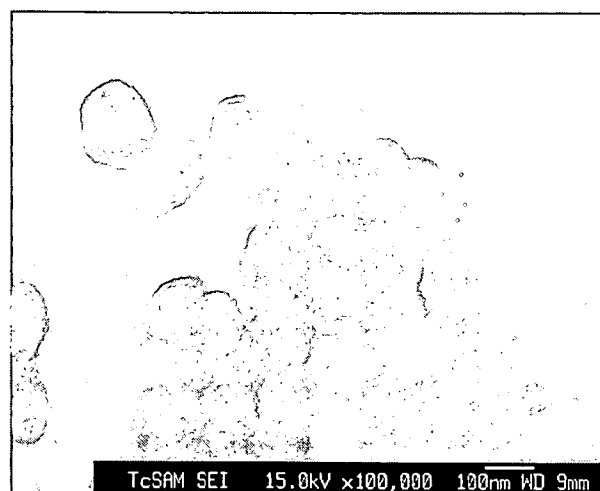
**Figure 5** UV- vis spectra of silver core-gold shell of various sizes and thicknesses of cores and shells.



**Figure 6** TEM images of silver core-gold shell particles.



AgNP10-11  
(55 nm core, ~15 nm shell)



AgNP6-14  
(75 nm core, ~15 nm shell)

**Figure 7** FE-SEM images of silver core – gold shell particles.



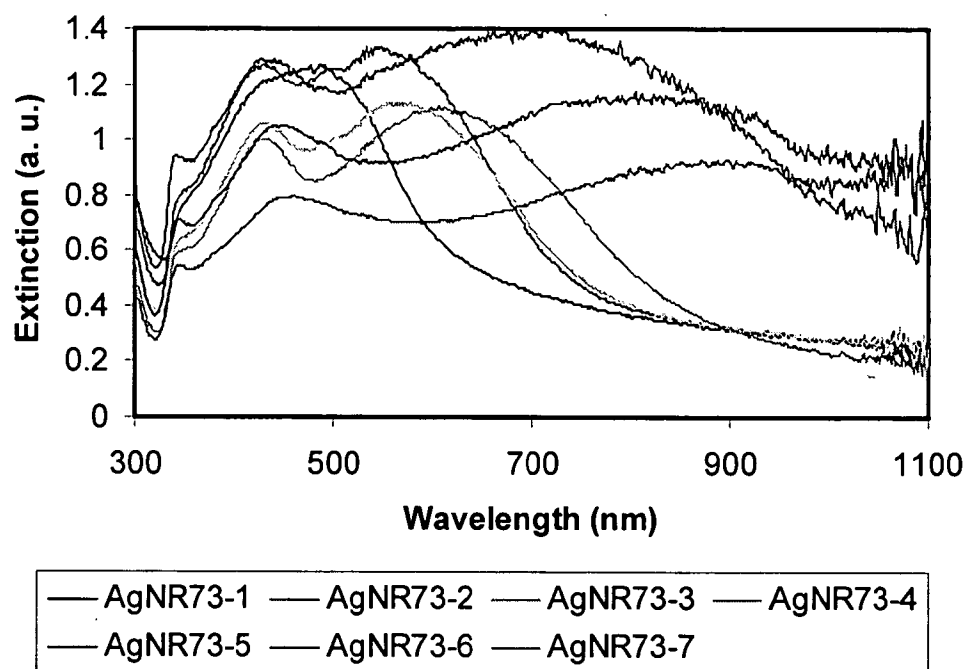
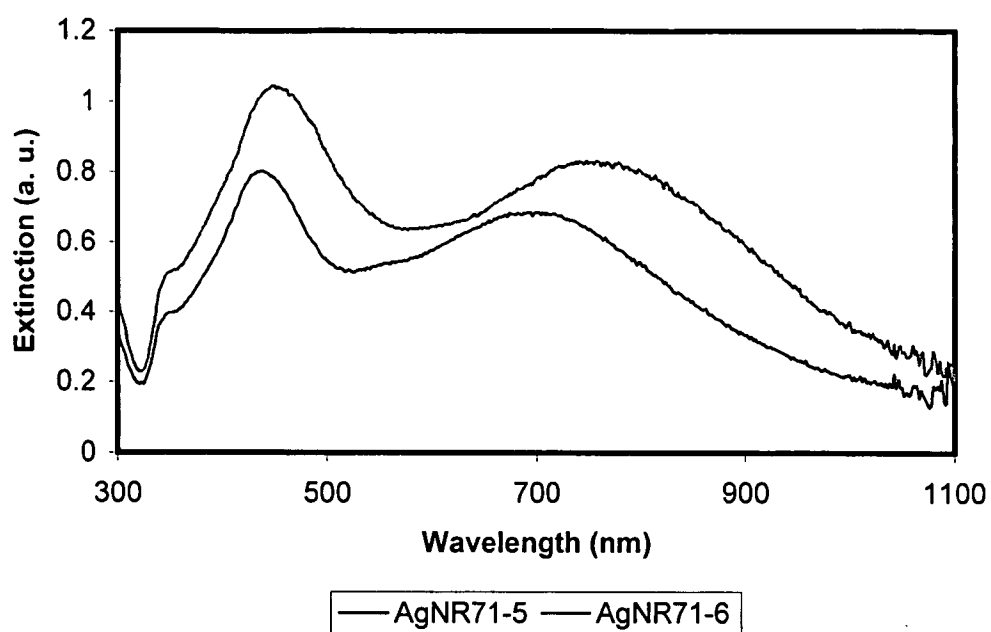
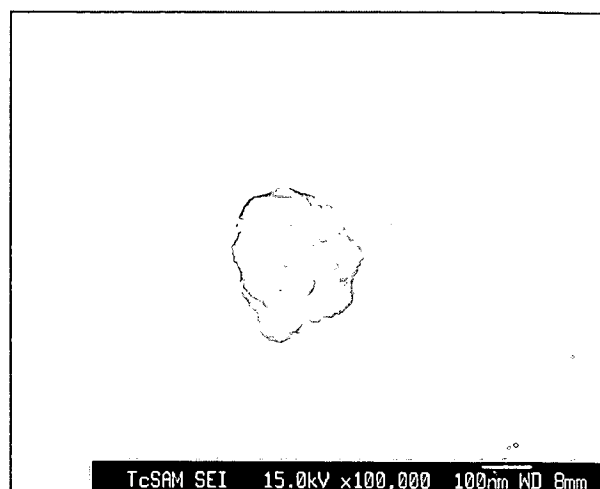
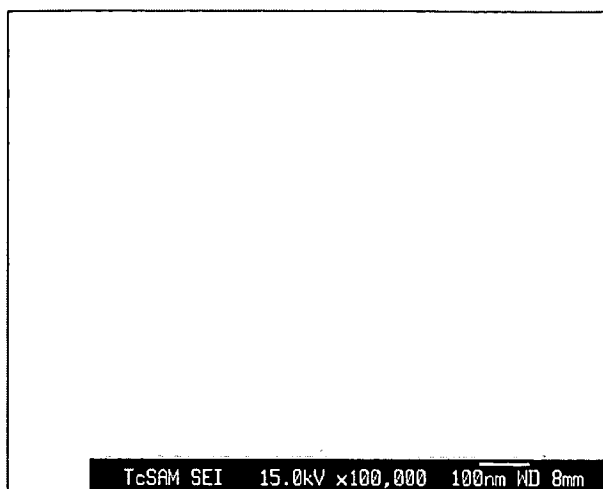
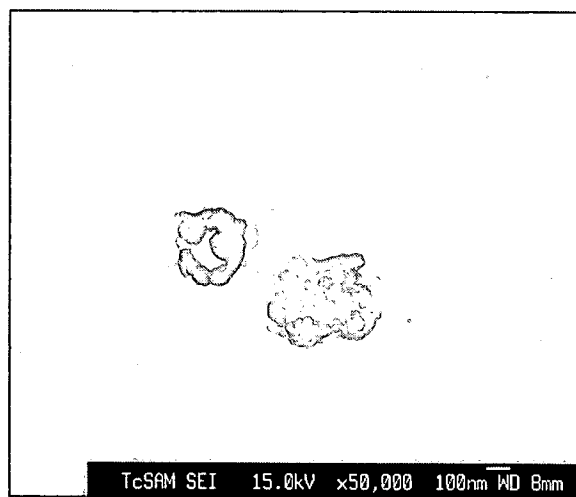
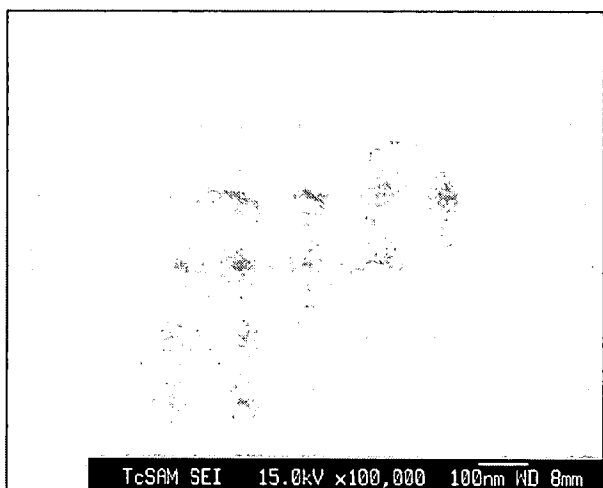


Figure 8 UV-vis spectra of silica core-silver nanorod particles.



(a) Silver nanorod 71-5

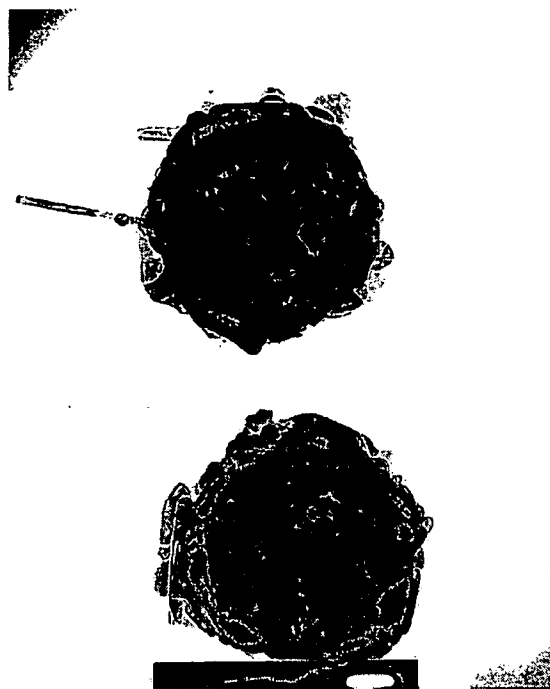


(b) Silver nanorod 71-6

**Figure 9** FE-SEM images of silica core–silver nanorod particles.

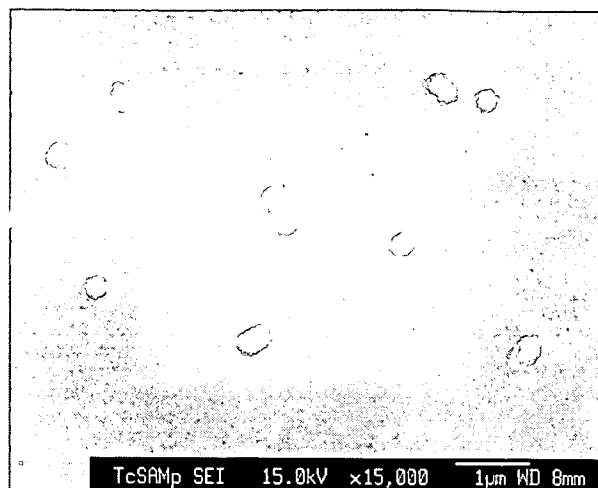
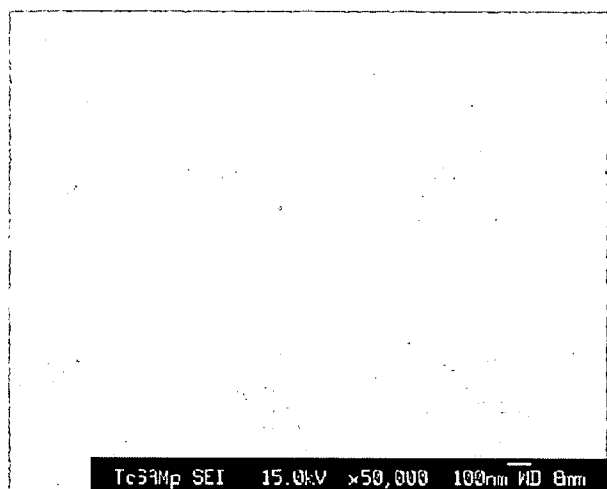


(a) Silver nanorod 73-1

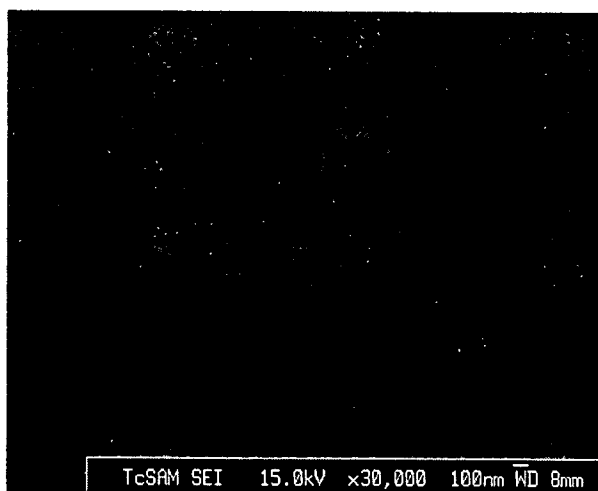
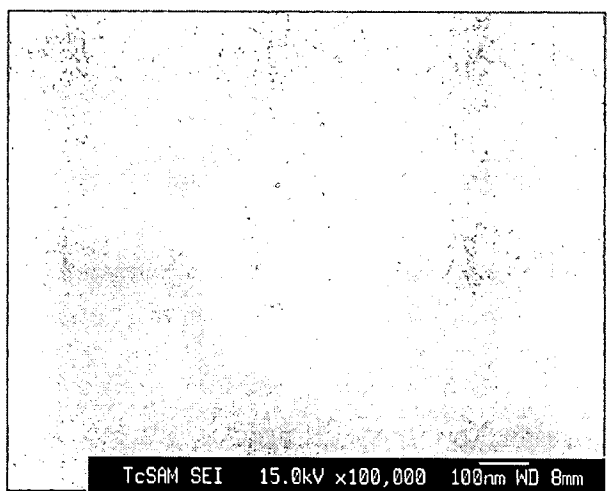


(b) Silver nanorod 73-6

**Figure 10** TEM images of silica core–silver nanorod particles.

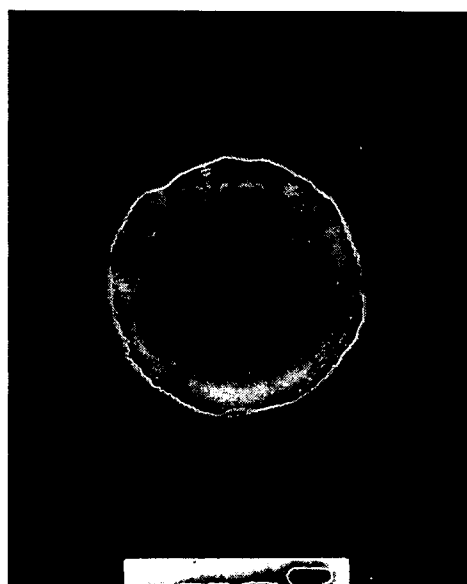
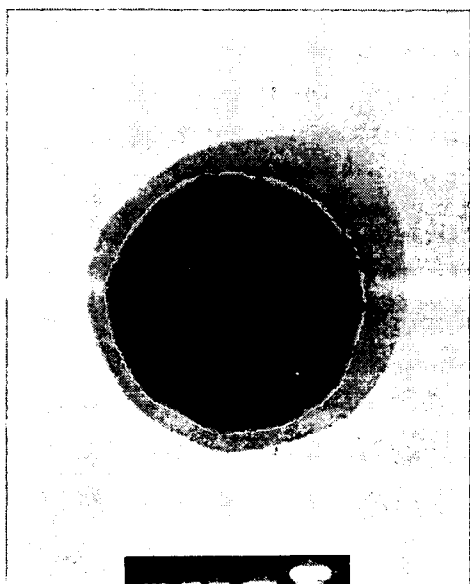


(a) Discrete hydrogel-coated gold nanoshell (120 nm core) particles



(b) Discrete hydrogel-coated gold nanoshell (100 nm core) particles

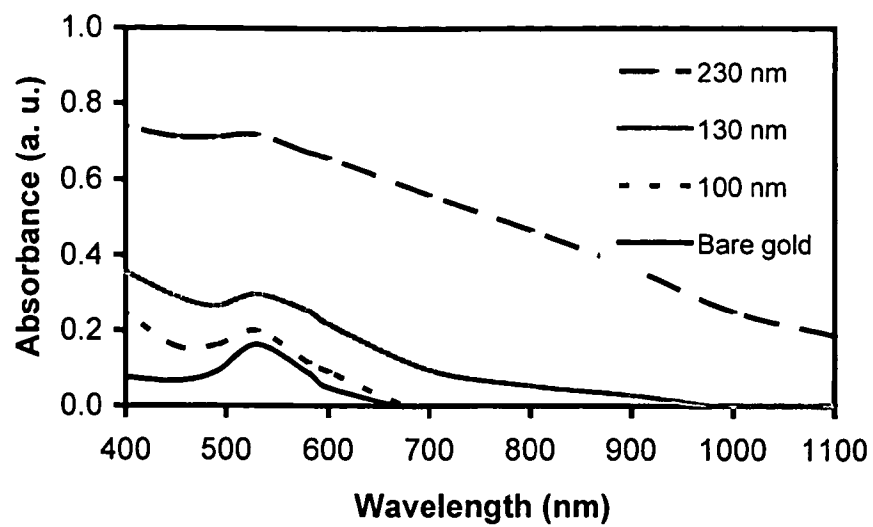
**Figure 11** FE-SEM images of discrete hydrogel-coated gold nanoshell particles.



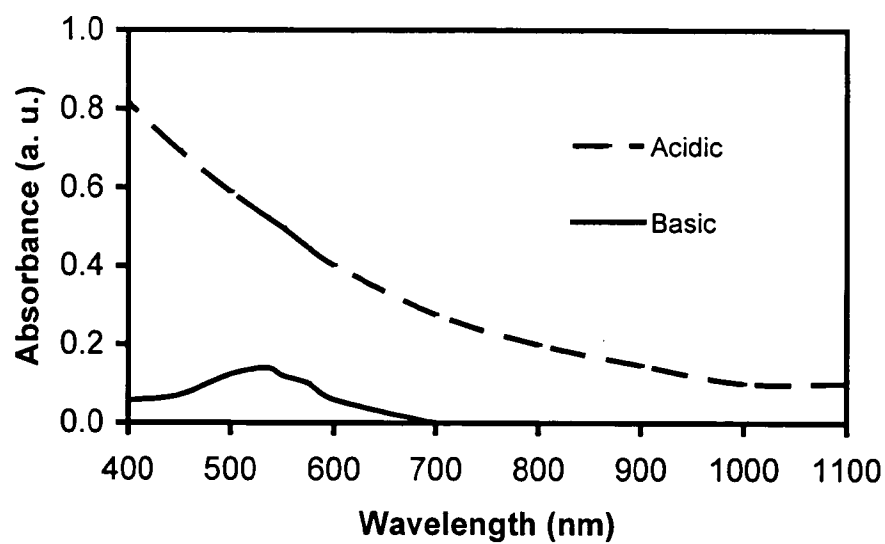
(b) Discrete hydrogel-coated gold nanoshell (100 nm core) particles.

**Figure 12** TEM images of discrete hydrogel-coated gold nanoshell particles.



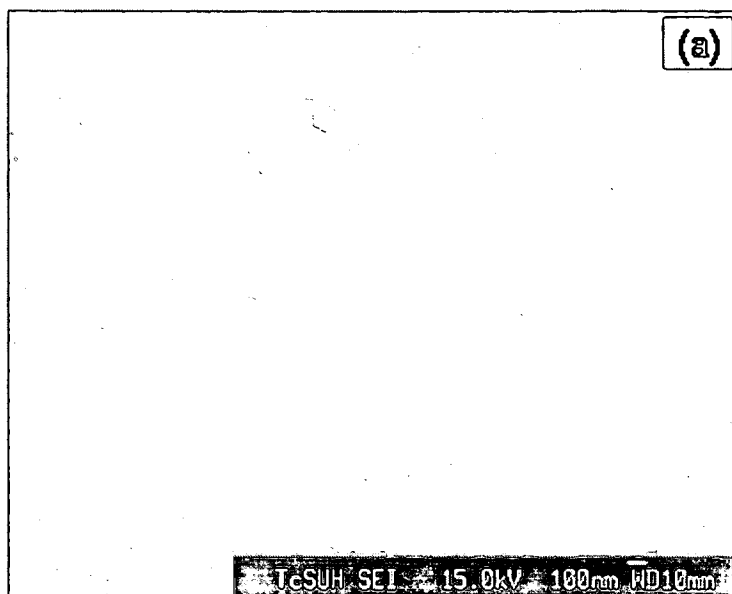


(a) Neutral



(b) Acidic or basic

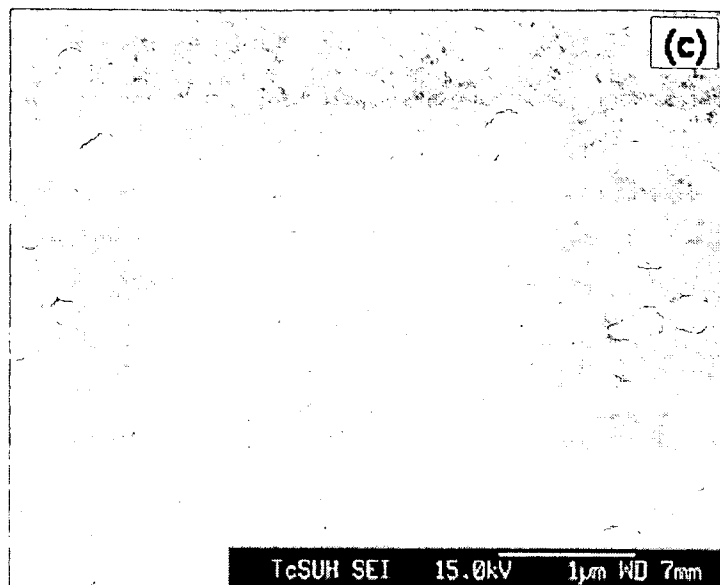
**Figure 14** Absorbance spectra of hydrogel-coated gold shell particles in (a) neutral and (b) acidic or basic media.



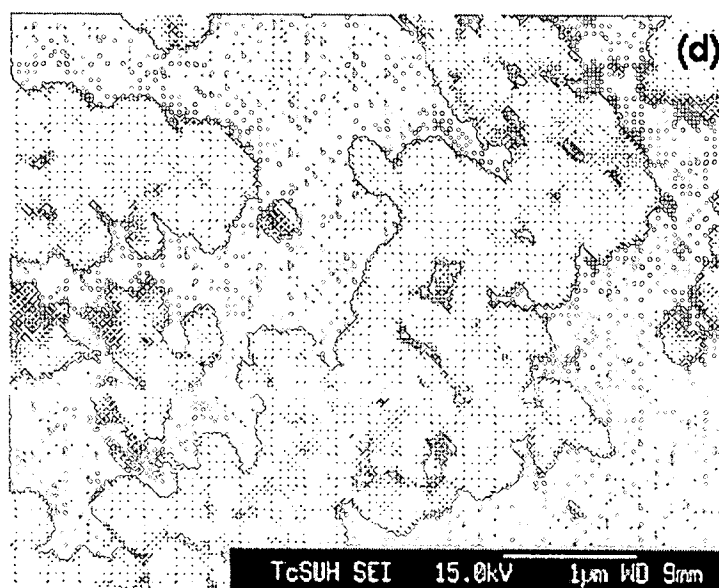
(b) Hydrogel-coated gold nanoparticles (~100 nm)

**Figure 15** FE-SEM images of nanoparticles of various sizes.  
(Figures 15 c and d continue on the following page.)





(c) Hydrogel-coated gold nanoparticles (~130 nm)



(d) Hydrogel-coated gold nanoparticles (~230 nm)

**Figure 15 c and d** FE-SEM images of nanoparticles of various sizes continued.

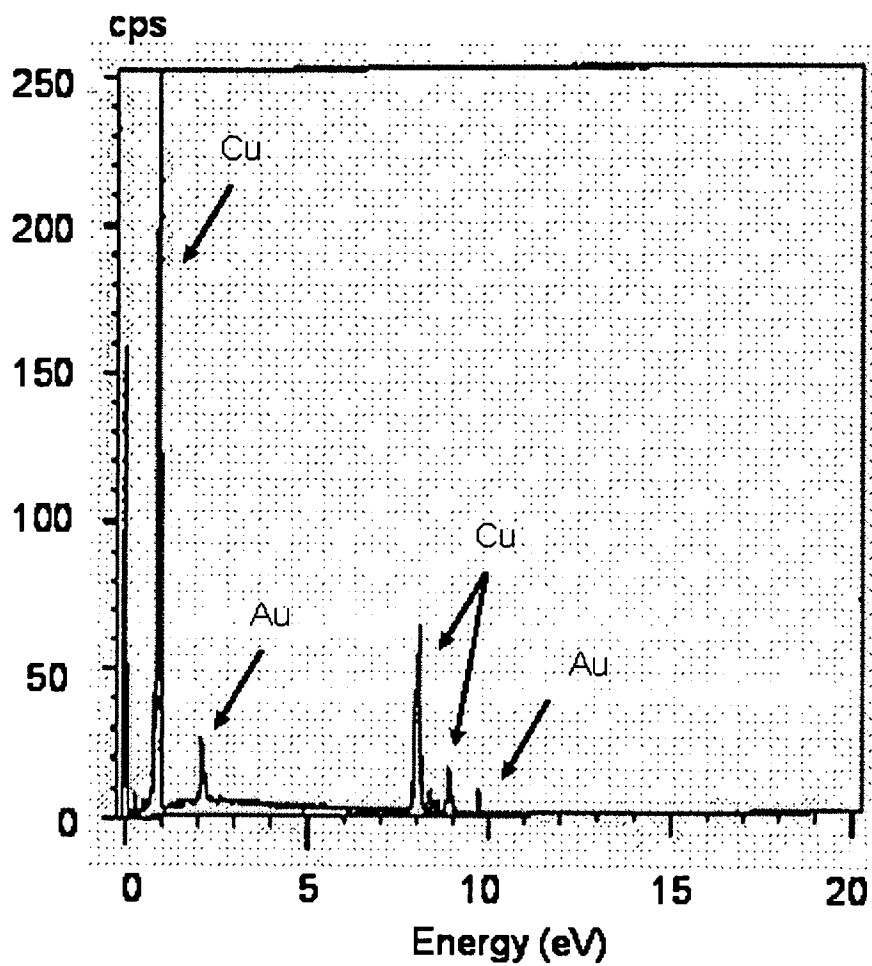
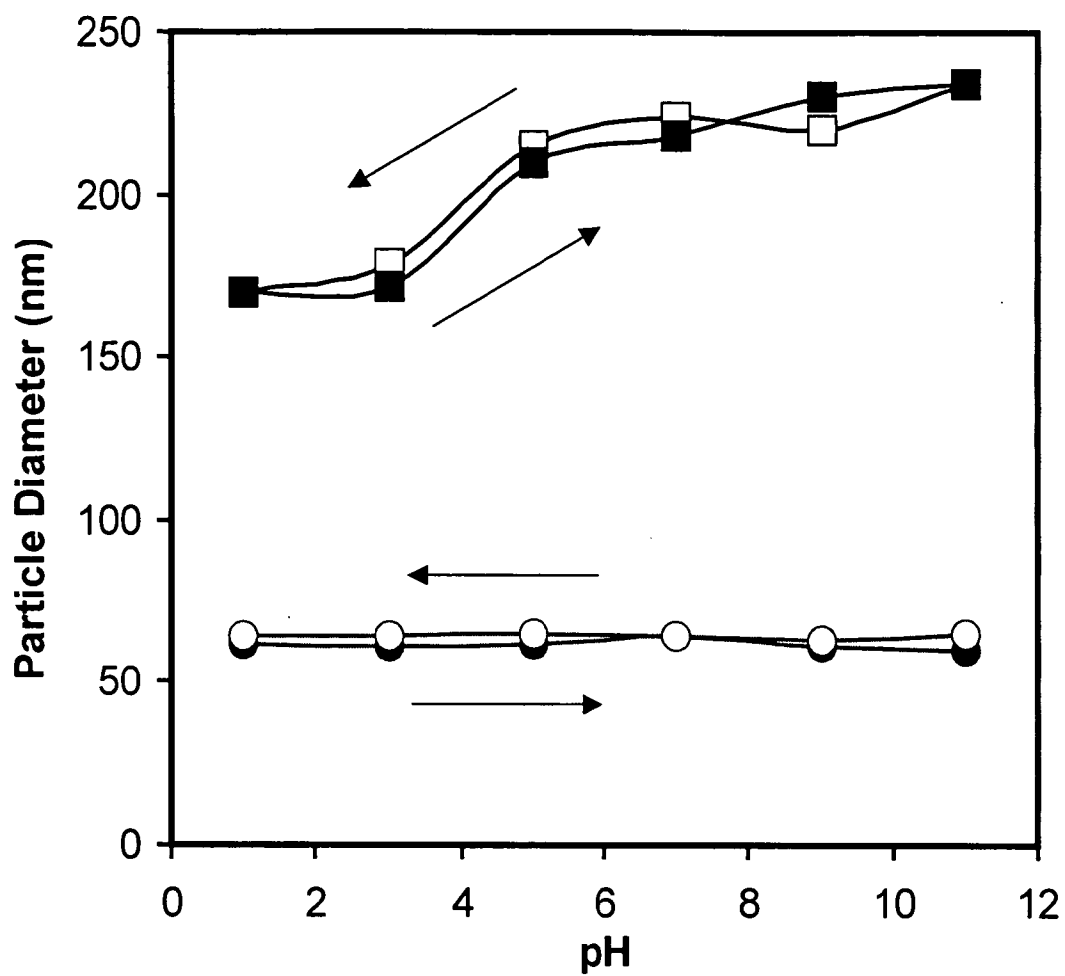
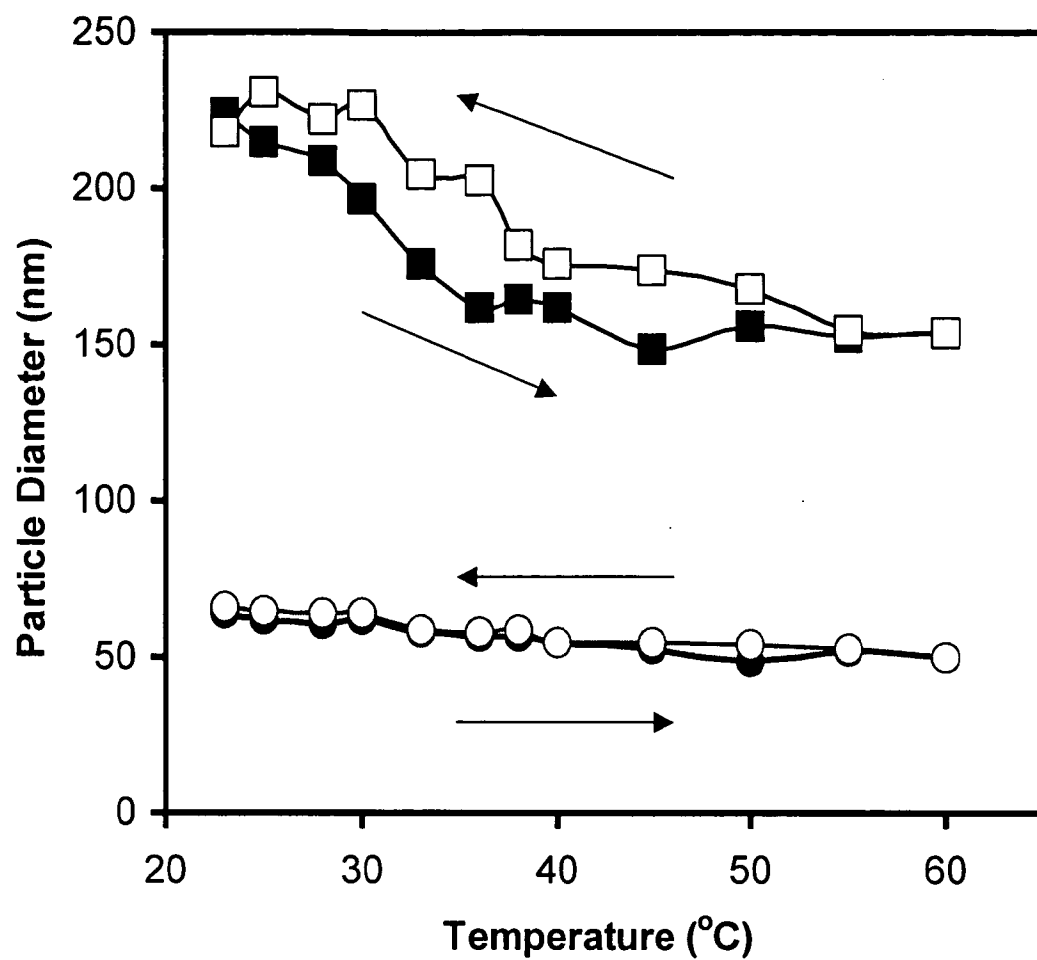


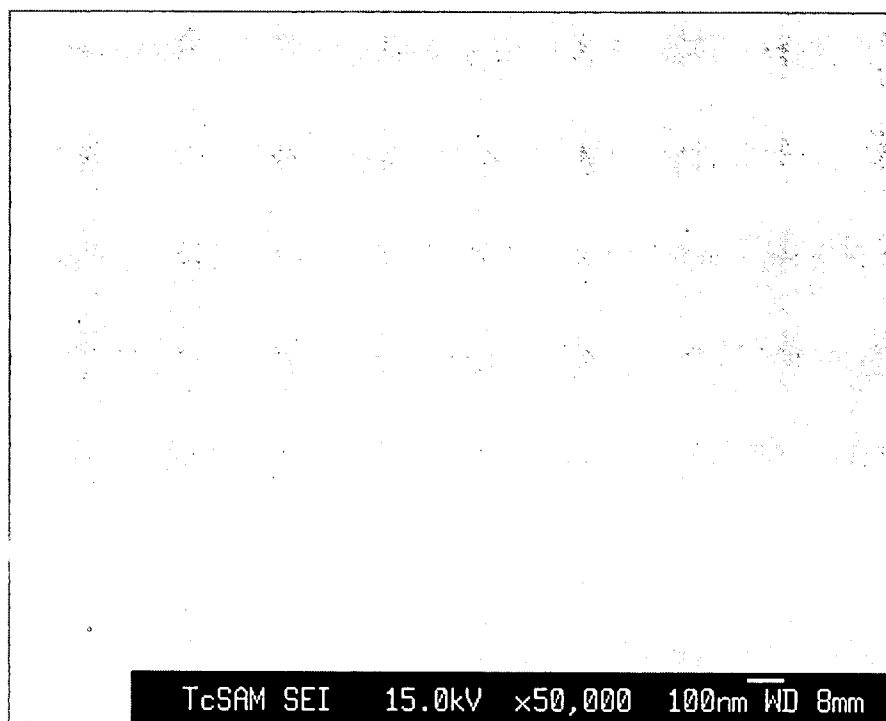
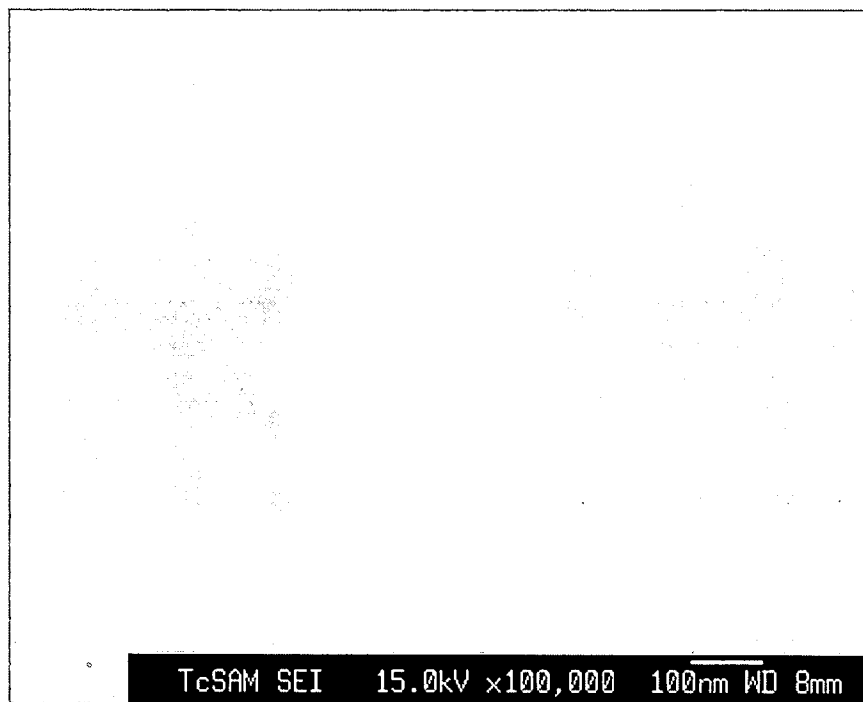
Figure 16 EDX spectrum of hydrogel-coated gold nanoparticles.



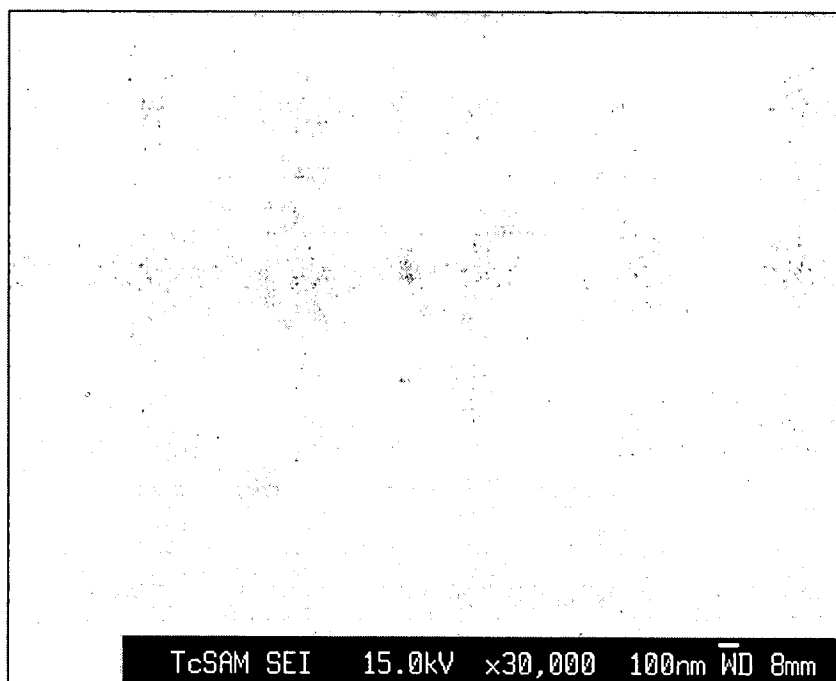
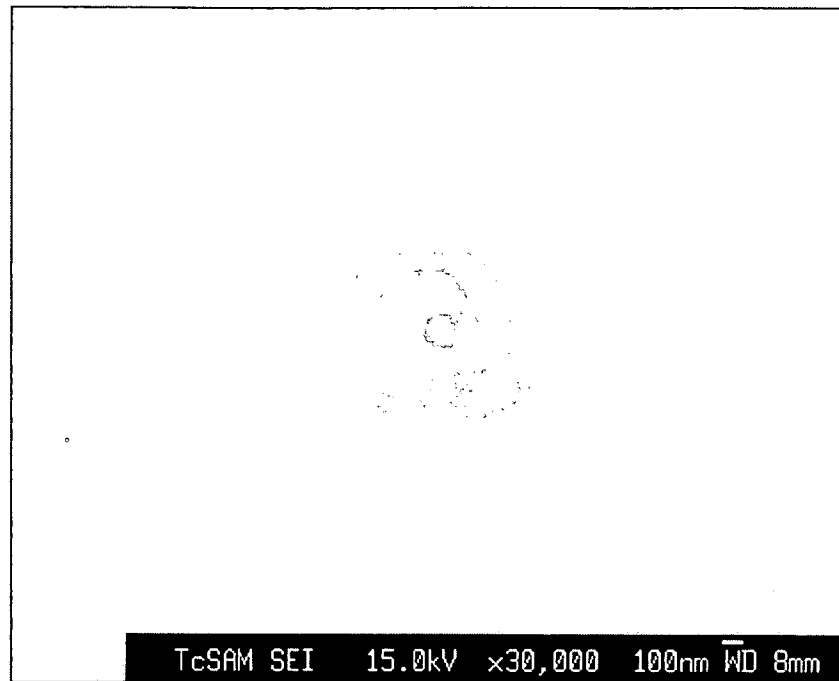
**Figure 17** A plot of particle size verse pH for bare gold nanoparticles and hydrogel-coated gold nanoparticles



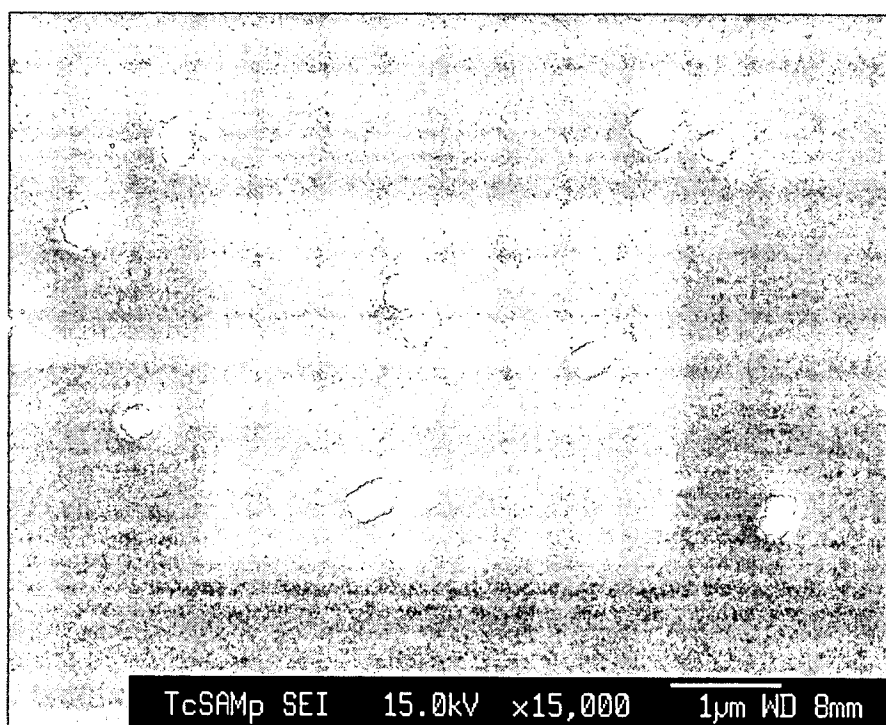
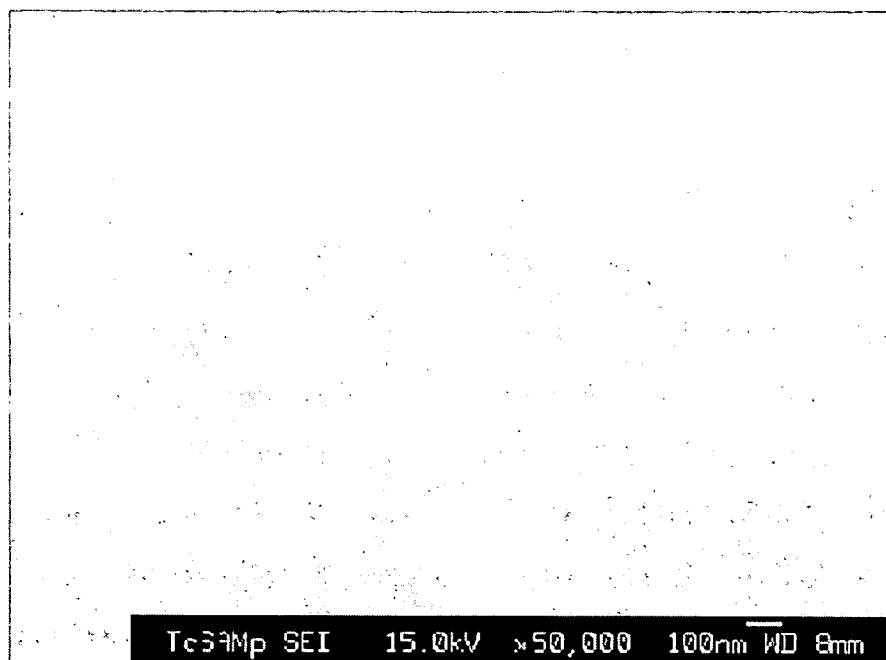
**Figure 18** A plot of particle size verses temperature for bare gold nanoparticles and hydrogel-coated gold nanoparticles.



**Figure 19** FE-SEM image of hydrogel-coated gold nanoshells (nanoshell core ~ 100 nm with a thin coating).



**Figure 20** FE-SEM image of hydrogel-coated gold nanoshells (nanoshell core ~ 100 nm with a thick coating).



**Figure 21** FE-SEM image of hydrogel-coated gold nanoshells (nanoshell core ~ 120 nm with a thin coating).